The Comparative UVB Photobiology of *Phaeocystis antarctica* and Selected Species of Antarctic Marine Diatoms

by

Andrew T. Davidson (B.Sc. Hons.)

Submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy

University of Tasmania

May 1996

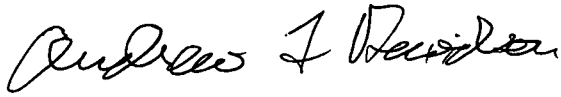
Declaration

Except as stated herein, this Thesis contains no material which has been accepted for the award of any other degree or diploma in an tertiary institution and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

(Andrew T. Davidson)
23 September 1996

Authority of Access

This thesis may be made available for loan and limited copying in accordance with the copyright act 1968



(Andrew T. Davidson)
23 September 1996

ABSTRACT

Stratospheric ozone protects the earth's surface from short wavelength UVB radiation. Over Antarctica, ozone concentrations presently fall to less than 30% of pre-ozone hole values during spring and ozone depletion persists into January and February. UVB reduces phytoplankton growth, primary production and survival in the upper 10–20 meters of the water column. Shallow phytoplankton blooms in the sea ice and marginal ice zone, which are responsible for much of the primary production in the Southern Ocean, appear vulnerable to damage by increased UV-B radiation.

This thesis examines the effect of UVB radiation on Phaeocystis antarctica and selected species of Antarctic marine diatoms. The colonial stage in the life cycle of P. antarctica was found to possess high concentrations of UV-absorbing compounds but diatoms lacked significant concentrations of these compounds. Results from the laboratory using artificial sources of UVB, and obtained under natural solar radiation at Davis Station, Antarctica, showed marked interspecific differences in the tolerance of Antarctic phytoplankton to UVB. Survival, primary production and growth of colonial P. antarctica when exposed to UVB was compared with that of the flagellate stage in the life cycle of this alga and selected diatom species. Unexpectedly, laboratory experiments showed that the diatoms, which lacked UV absorbing compounds, survived UVB irradiances three to five times higher than those that caused mortality in colonial stage P. antarctica. Exposure of diatoms to PAR and UVA reduced their survival. However, low UVB irradiances increased their survival to levels similar to PAR exposed control treatments. Thus, UVB may be involved in photorepair mechanism for UVA induced damage. Competition experiments, using cultures of motile and colonial P. antarctica together with diatoms, were exposed to natural Antarctic irradiance at Davis Station. These showed direct evidence of UVB induced changes in phytoplankton species composition. Growth of the colonial stage of P. antarctica was enhanced at the expense of diatoms. Thus, laboratory experiments which showed that the colonial life stage of P.

antarctica was the most susceptible species to UVB induced mortality were not indicative of its relative success at natural Antarctic sublethal fluxes.

P. antarctica is important in Antarctic waters for several reason. Its blooms are, pivotal in determining trophic function, poorly grazed by metazooplankton, contribute little to the flux of carbon to deep water and are known to release high concentrations of dimethylsulfide to the atmosphere which act as cloud condensation nuclei. Results reported here indicate the potential for altered trophodynamics, vertical carbon flux and global albedo as a result of Antarctic ozone depletion.

ACKNOWLEDGMENTS

I wish to extend my heartfelt gratitude to Dr. Harvey Marchant of the Australian Antarctic Division for his friendship, support, guidance, advice, patience, accessibility, enthusiasm, perseverance, competence and for aiding the development of both this research and myself. I also wish to thank Dr. Andrew McMinn from the Institute of Antarctic and Southern Ocean Studies at the University of Tasmania for his friendly advice and guidance with the research and thesis. Thank you to all of those who assisted both morally and physically during the research. For their help during field operations in Antarctica I wish to thank Jeff Hunt, Paul Synnot, Lionel Whitehorn, Peter Sprunk, Danny Eslake and Fiona Scott. I thank Jim Dalpont from CSIRO for organic carbon analyses, Jane Masterson who worked with me for one month as a work experience student and Deborah Bramich whose honours project contributed significantly to my attitudes towards the research and whose friendship contributed significantly to my mental wellbeing. I also wish to thank all those who have supplied advice on methods including Dr. G. Hosie and Dr. de la Mare with statistics and S. Wright with HPLC of UV absorbing compounds. For their aid with some figures contained in this thesis I thank John Cox and the staff in the Antarctic Division's Multi Media branch. Many thanks also to Dr. Gustaff Hallegraeff, Dr. Shirley Jeffrey, Dr. David Garrison, Dr. John Parslow, Prof. Pat Quilty, Dr. Steve Nicol and Deborah Bramich for their constructive criticism of manuscripts prior to submitting them for publication. Finally, I wish to thank my wife Kathryn, and children James and Erica for their support, abiding my preoccupation and accepting my absences at work and in Antarctica.

INTRODUCTION

Stratospheric ozone depletion over Antarctica since the mid 1970s has enhanced short wavelength solar UVB radiation reaching the Earth's surface (Frederick & Snell 1988, Lubin et al. 1980). These wavelengths are responsible for reducing survival, growth and primary production of marine phytoplankton in the upper water column (Holm-Hansen et al. 1989, Karentz 1989, Smith et al. 1992). Species and life stages differ in their growth and survival response to UVB radiation (Calkins & Thordardottir 1980, Karentz et al. 1991a, Marchant et al. 1991). Karentz et al. (1991a) noted that very little is known of the responses of individual Antarctic phytoplankton species to UVB exposure. Such information is integral to understanding the potential effect of enhanced UVB irradiances on Antarctic phytoplankton. Changes in phytoplankton survival, production and species composition may significantly alter trophodynamics and biogeochemical process in Antarctic waters. The subject of this thesis is "The comparative UV photobiology of Phaeocystis antarctica and selected species of Antarctic marine diatoms". This research was begun in 1991 as a part-time Doctor of Philosophy and was undertaken to examine the UVB photobiology of important contributors to the Antarctic phytoplankton assemblage; their survival, production, growth and UVB induced changes in species composition.

The first chapter of the thesis introduces the topic of UVB and phytoplankton photobiology. A brief introduction to Antarctic phytoplankton is provided in Chapter 2. The biology and ecological role of Phaeocystis, is examined in the third chapter. Chapter 4 reports research conducted at Davis Station, Antarctica, to examine the importance of P. antarctica as a determinant of protistan community structure and function. Results underline the pivotal role played by blooms of P. antarctica in determining protistan and bacterial community dynamics. Subsequent chapters present experiments conducted to examine the UVB photobiology of these organisms. Laboratory research is presented that investigates the survival and UV absorbing pigment complement of unialgal cultures of

Phaeocystis antarctica (Chapter 5) and selected diatom species (Chapter 6). The effect of natural Antarctic UV irradiance on growth, production and survival are then reported for unialgal cultures of P. antarctica and diatoms (Chapter 7) and for mixed species competition experiments that examine the effect of UVB on phytoplankton species composition (Chapter 8). Finally, conclusions are drawn from our research examining the potential impacts of enhanced UVB irradiances on trophodynamics and biogeochemical processes in Antarctic waters (Chapter 9).

Much of the material contained within this thesis has already been published (see Appendix 1 for reprints & below for publication list). Reviews of the genus Phaeocystis for Progress in Phycological Research and a compilation of our UVB research for the American Geophysical Union's Antarctic Research Series were submissions invited by the editors of these volumes namely, Prof. Frank Round and Drs. Polly Penhale and Susan Weiler respectively. As material has been published since 1991 the referencing, content and context of the Thesis text has been up-dated to reflect the current state of knowledge.

PhD Related Publications

Marchant HJ, Davidson AT, Kelly GJ (1991) UV-B protecting pigments in the marine alga Phaeocystis pouchetii from Antarctica. Mar Biol 109: 391-395

Marchant HJ, Davidson AT (1991) Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. In: Weller G. Wilson CL, Severin BAB(eds) Proceedings of the international conference on the role of polar regions in global change. Geophysical Institute, Fairbanks, p 397-400

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

Davidson AT, Marchant HJ (1992) The biology and ecology of Phaeocystis (Prymnesiophyceae). In: Round FE, Chapman DJ (eds) Progress in phycological research. vol 8, Biopress, Bristol, p 1-46

Davidson AT, Bramich D, Marchant HJ, McMinn A (1994) Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms. Mar Biol 119: 507-515

Davidson AT, Marchant HJ (1994) The impact of ultraviolet radiation on Phaeocystis and selected species of Antarctic marine diatoms. In: Weiler CS, Penhale PA (eds.) Ultraviolet radiation in Antarctica: measurement and biological effects. Antarctic Research Series, vol 62, American Geophysical Union, Washington, DC, p 160-187

Davidson AT, Marchant HJ (1994) Comparative impact of in situ UV exposure on productivity, growth and survival of Antarctic Phaeocystis and diatoms. Proc NIPR Symp Polar Biol 7: 53-69

Davidson AT, Marchant HJ, de la Mare W (1996) Direct evidence that natural UVB exposure changes Antarctic phytoplankton species composition. Aquatic Microbiol Ecol 10: 299-305

CHAPTER 1

The impact of UV radiation on Antarctic phytoplankton

1. INTRODUCTION

Photosynthesis is the means by which new energy enters the biological system (Kirk 1983, Prézelin et al. 1991). It is also a principal determinant of marine processes including food web dynamics and biogeochemical cycling of compounds, that affect everything from marine chemistry to regional and global weather patterns (SCOPE 1993). Many of the biological, physical and chemical process in the marine environment determined by light are dependent on its spectral characteristics. Marine ecosystems have evolved over millennia to occupy this environment. However, in the brief period of two decades, anthropogenic chlorofluorocarbons and halons have inadvertently contributed to a marked depletion of stratospheric ozone (O_3); the Earth's atmospheric protection against high energy, biologically damaging, short wavelength ultraviolet-B radiation (UVB) (280 - 320 nm) (eg Crutzen 1992, Jones & Shanklin 1995, Madronich 1995, von der Gathen et al. 1995). Assuming full international compliance with the Montreal protocol and subsequent amendments regarding phasing out production of ozone depleting compounds, stratospheric ozone will not return to pre-ozone hole concentrations until around the middle of next century (eg. Madronich 1995).

The impact of increased UVB radiation on marine organisms is uncertain. UVB is strongly attenuated with depth by physical and biological processes (see section 2) and mediated by tolerance mechanisms (see section 5). Amongst a rapid and fast-growing literature on biological effects of UV radiation, the impact of UVB on phytoplankton having received by far the most scientific attention (Marchant 1994). However, estimates of the UVB–induced decline in primary production in Antarctic waters varies enormously (Voytek 1989, 1990, Smith et al. 1992, Behrenfeld et al. 1993, Cullen et al. 1992,

Helbling et al. 1994, Schofield et al. 1995). There is also no clear evidence of UVB–induced changes in phytoplankton species composition since ozone depletion began (McMinn et al. 1994).

There is overwhelming scientific evidence that UVB radiation is damaging to marine organisms (UNEP 1989, 1991, USEPA 1987). Enhanced UVB is likely to cause subtle community-level changes in marine communities (Vincent & Roy 1993) in an ecosystem in which the biotic and abiotic environment are highly variable in space and time (eg. Smith et al. 1988). That UVB induced changes in the marine environment are difficult to detect belies their importance. Such changes may influence biological process at all trophic levels (Vincent & Roy 1993) and significantly alter biogeochemical cycles that are involved in determining global climate (Marchant & Davidson 1991). Therefore, anticipation of the consequences of ozone depletion and investigations of the capacity of marine organisms to tolerate UVB exposure are extremely important (Worrest & Häder 1989).

This chapter primarily examines the direct impact of UVB on phytoplankton and indirect influences of UVB as a result of interactions with their biotic and abiotic environment. It provides a brief overview of the marine light environment, the biological effects of UVB radiation and protective mechanisms that organisms employ to mitigate against these wavelengths. The responses of phytoplankton and other members of marine biota to UVB are then examined and conclusions drawn regarding the possible impact of ozone depletion on Antarctica phytoplankton.

2. LIGHT IN THE MARINE ENVIRONMENT

The reliance of marine photosynthetic organisms on solar energy restricts them, and many organisms directly dependent on them as a source of nutrition, to the upper illuminated layers of the world's oceans. This obligate requirement for light also makes them vulnerable to changes in solar UVB radiation (Worrest & Häder 1989). The light

climate in the marine environment is heterogeneous. Changes in latitude, cloud cover, and seasonal and diurnal fluctuations in solar irradiance also influence the amount and wavelength structure of incident light at the sea surface (eg. Hardy & Gucinski 1989, El-Sayed et al. 1990, Gautier et al. 1994). Mutual shading by phytoplankton, scattering and absorption by particulate and dissolved material (eg Smith & Baker 1979, 1989), back-reflectance from ice, snow, and the sea surface (Karentz 1991) further influence the light climate. The ultrastructural complexity of cells also causes backscatter and attenuation of light (Kitchen & Zaneveld 1992). Light wavelengths are differentially attenuated with depth; short and long wavelengths being absorbed more strongly than green and blue Jerlov (1950). UVB is strongly absorbed by these physical and biological factors but in Antarctic waters UVB may penetrate seawater to depths in excess of 50 m (Gieskes & Kraay 1990, Karentz & Lutze 1990, Smith et al. 1992) and biological effects could be detected to 20-30 m (Karentz & Lutze 1990). Vertical mixing may then carry planktonic organisms through light climates from near-surface to darkness. Thus, determining the biological significance of increased UVB is extremely difficult and is further exacerbated by species specific differences in physiological ability to tolerate UVB exposure and damage (see Chapter 1 sections 5 & 6).

3. OZONE AND UVB RADIATION

The unique conditions of an enclosed airmass over Antarctica in the polar vortex, low temperature and polar stratospheric clouds has meant that anthropogenic ozone depleting compounds most severely deplete ozone over Antarctica (Hoffman 1989). Depletion of stratospheric ozone (Fig. 1A) over Antarctica was first recognised in 1985 (Farman et al. 1986), approximately a decade after it began. The "ozone hole" was coined to describe the rotating, elliptically shaped region of depleted ozone that extended from the pole to around 60 - 70° S (Brasseur 1987, Solomon 1990). During spring, stratospheric ozone concentrations over Antarctica presently fall to less than 30% of pre-ozone hole values area approximately 20% greater than Australia (Stolarski et al. 1992). As a result, coastal

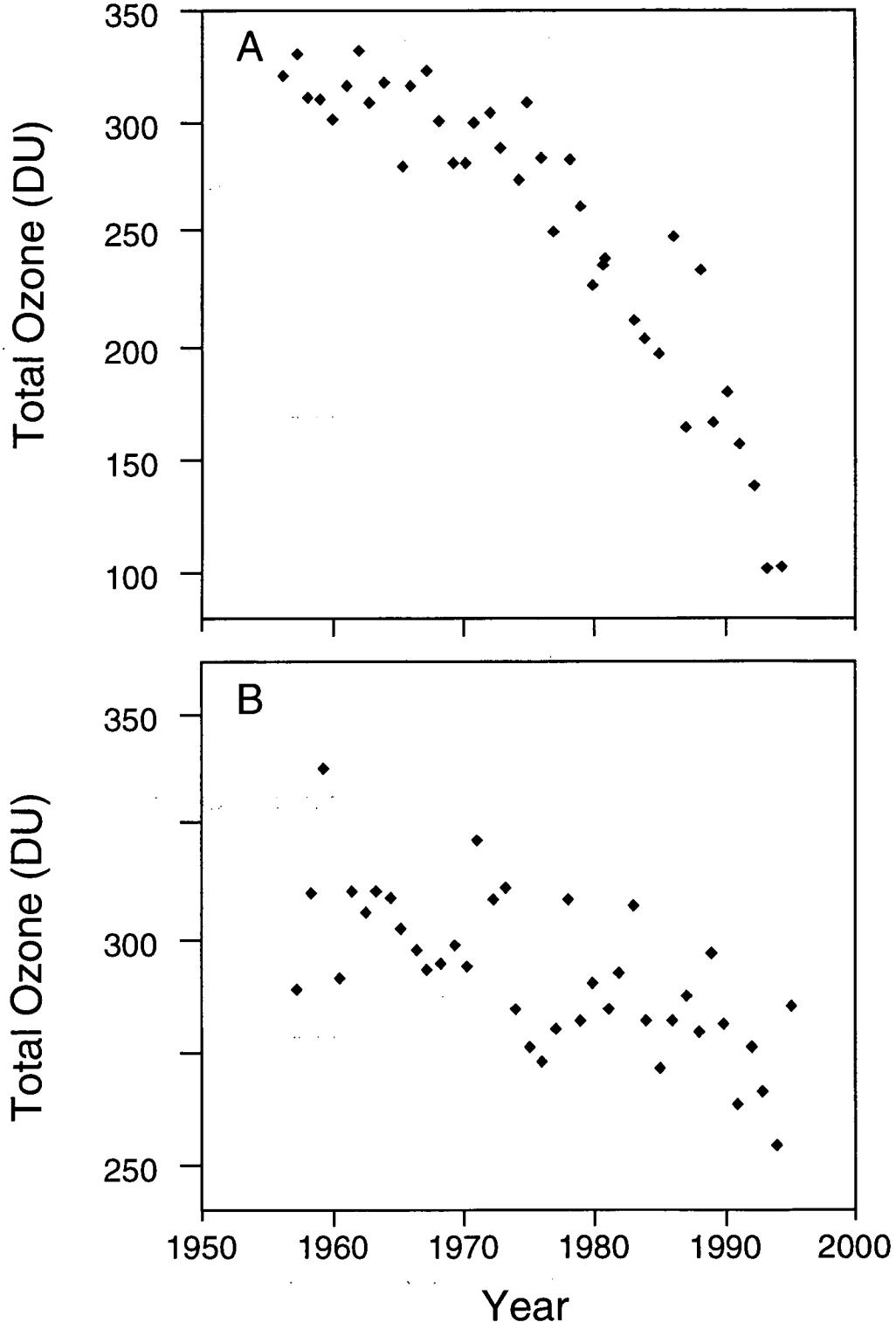


Fig. 1. Total ozone observed over Halley, Antarctica. (A) October mean total ozone from 1956 to 1994 and (B) monthly mean total ozone from 1957 to 1995 in February (redrawn from Jones & Shanklin 1995).

Antarctic springtime UVB irradiance is at least as high as that at the summer solstice (Frederick & Snell 1988, Lubin et al. 1989). Breakdown of the polar vortex in November causes ozone depleted stratospheric air to be transported to mid-latitudes (Atkinson et al. 1989). However, ozone depletion over Antarctica now persists into January and February (Jones & Shanklin 1995) (Fig. 1B). Persistence of this depletion over summer, together with the higher solar elevations around the summer solstice, result in 50-100% higher UV values than normally experienced (Frederick & Lubin 1994).

Concern is now growing about development of an ozone hole over northern polar regions and a generalised depletion of ozone at middle latitudes, thereby increasing UVB radiation over some of the Earth's most biologically productive and inhabited regions (Crutzen 1992, Stolarski et al. 1992, Seckmeyer & McKenzie 1992, Lubin & Jensen 1995, Madronich 1995, von der Gathen et al. 1995). Furthermore, Jones & Shanklin (1995) report that ozone depletion has caused a decrease in stratospheric temperatures. This, they point out, would lead to an increase in stratospheric cloud, which acts as the reaction site for much of the ozone destruction, and further accelerate the rate of ozone depletion.

Tropospheric halogen concentrations are reportedly declining and concentrations in the stratosphere are expected to peak between 1997 and 1999 (Montzka et al. 1996). Assuming full compliance with international agreements (the Montreal Protocol and Copenhagen Amendment), ozone depletion is expected to persist until the middle of next century (Jones & Shanklin 1995, Madronich 1995). However, studies incorporating the explosive growth expected by newly industrialised economies in India and Asia suggest that the global potential for ozone destruction in 2030 would be twice that in 1990 (MacKenzie 1995).

UVA and UVB radiation constitute only some 4% and 0.8% respectively of the total surface incident solar energy on a cloud-free day (Vincent & Roy 1993). Stratospheric ozone depletion does not significantly increase UVA (320 - 400) or photosynthetically

active radiation (400 - 700 nm) (PAR) (eg. Behrenfeld et al. 1993). Only UVB irradiances are increased, particularly at the shorter wavelengths within this region of the spectrum (Fig. 2). Wavelengths less than 299 nm were not experienced in Antarctica before the appearance of the ozone hole. Surface incident UVB radiation now extends to 294 nm (Roy et al. 1994). At a column-ozone depletion of around 65%, light at 315 nm is expected to increase 2.2-fold while the increase at 305 nm is predicted to exceed 14-fold (Frederick & Snell 1988, Booth & Madronich 1994). Thus, the ratio of UVB: UVA and PAR is increased more than 2-fold (Smith et al. 1992).

4. BIOLOGICAL HAZARDS OF UVB EXPOSURE

Most severe biological effects of exposure to light are much greater at short wavelengths, rising precipitously and becoming less reversible at wavelengths below 350 nm (Smith & Baker 1989, Karentz 1991, Vincent & Roy 1993) (Fig. 3). Action spectra illustrate the increased damage to organisms or cellular processes elicited per unit of irradiance at decreasing wavelength (eg. Jones & Kok 1966, Caldwell 1981, Setlow 1974, Smith & Baker 1979, Cullen et al. 1992, Behrenfeld et al. 1993). Energy from UVB radiation is absorbed by nucleic acids, DNA, RNA and proteins including enzymes, histones, hormones, membrane components (Häder & Worrest 1991, Tevini 1993, Karentz et al. 1991a) and is dissipated in damaging photochemical reactions that effect the structure, function, regulation and multiplication of cells. UVB is also absorbed by photosynthetic pigments, disabling the electron transfer chain and damaging the catalytic site of water oxidation (Renger et al. 1989), damaging the reaction centre in photosystem II and bleaching the pigments themselves (Häder 1993). Inhibition of enzymes such as Rubisco, ATP-synthase and those involved in biosynthesis of photosynthetic pigments, including the chlorophylls, further impairs primary production (Strid et al. 1990). Chromophore molecules within cells act as photosensitizers on absorption of a high energy UVB photon. Excess excitation energy from this absorption can form superoxides that destroy membranes and other cellular components (Häder 1993, Tevini

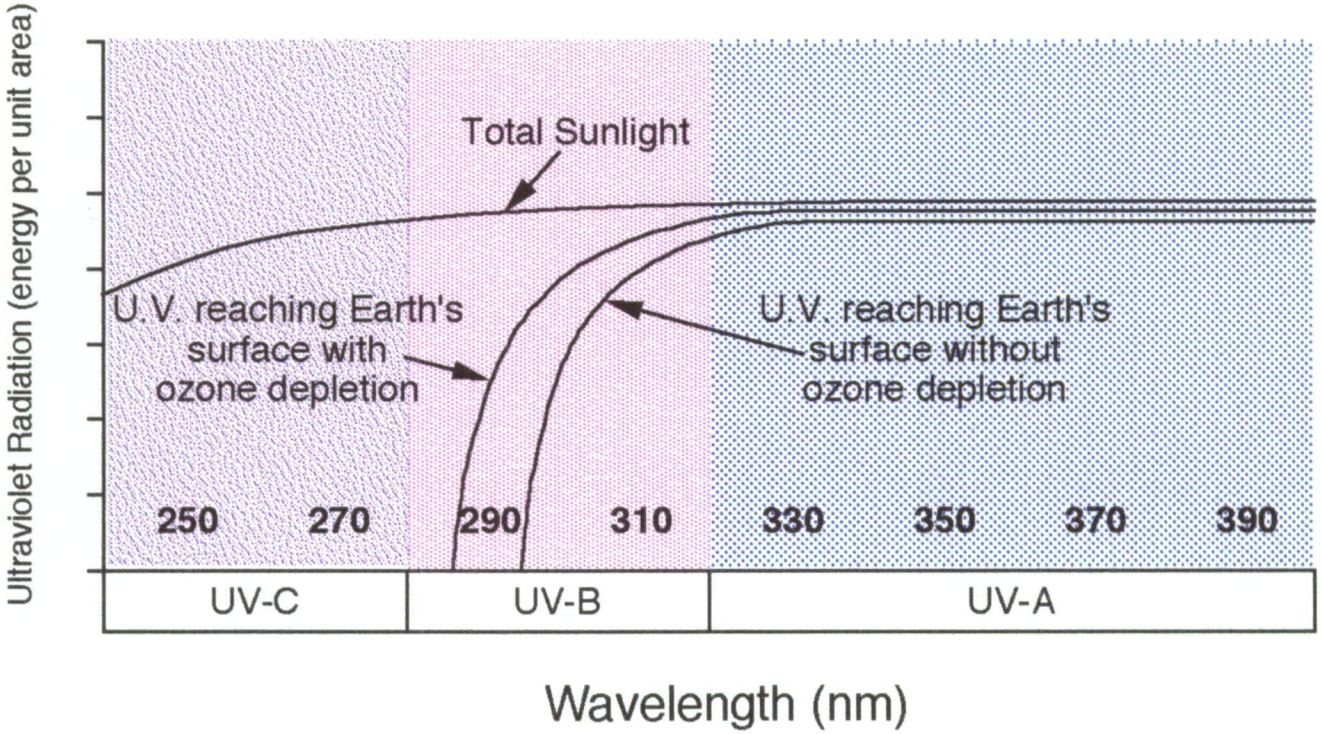


Fig. 2. UVA is only slightly attenutated by the atmosphere, while UVB is greatly attenuated. UVC is completely blocked. Any decrease in ozone will lead to more and shorter wavelengths of UVB reaching the Earth's surface.

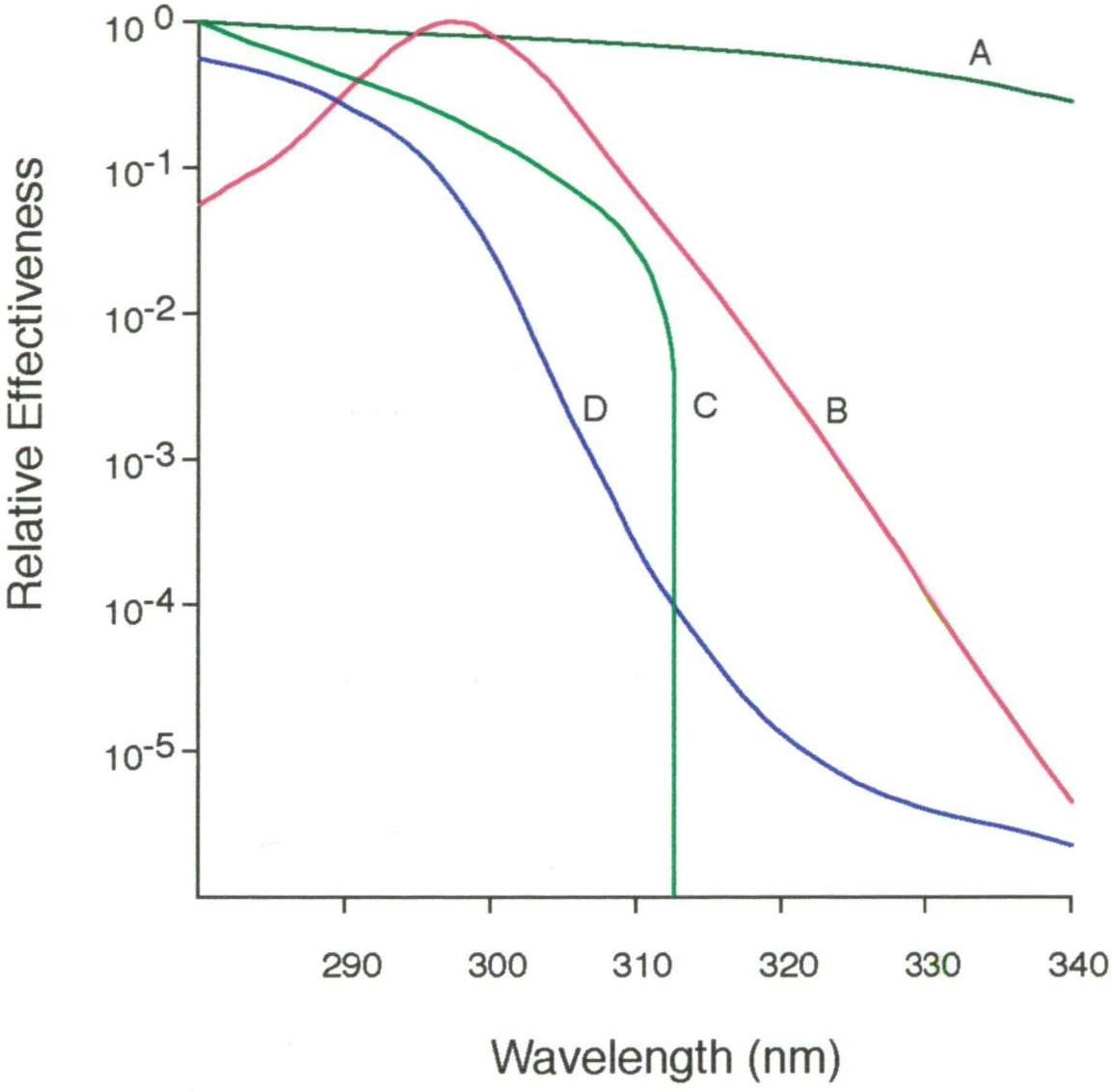


Fig. 3. Plots of several action spectra used as weighting functions for assessing the biological effects of UVB radiation. A, Photoinhibition action spectrum (Jones & Kok 1966); B, erythema; C, Caldwell's generalised plant spectrum; D, generalized DNA action spectrum (Setlow 1974) (redrawn from Worrest 1983).

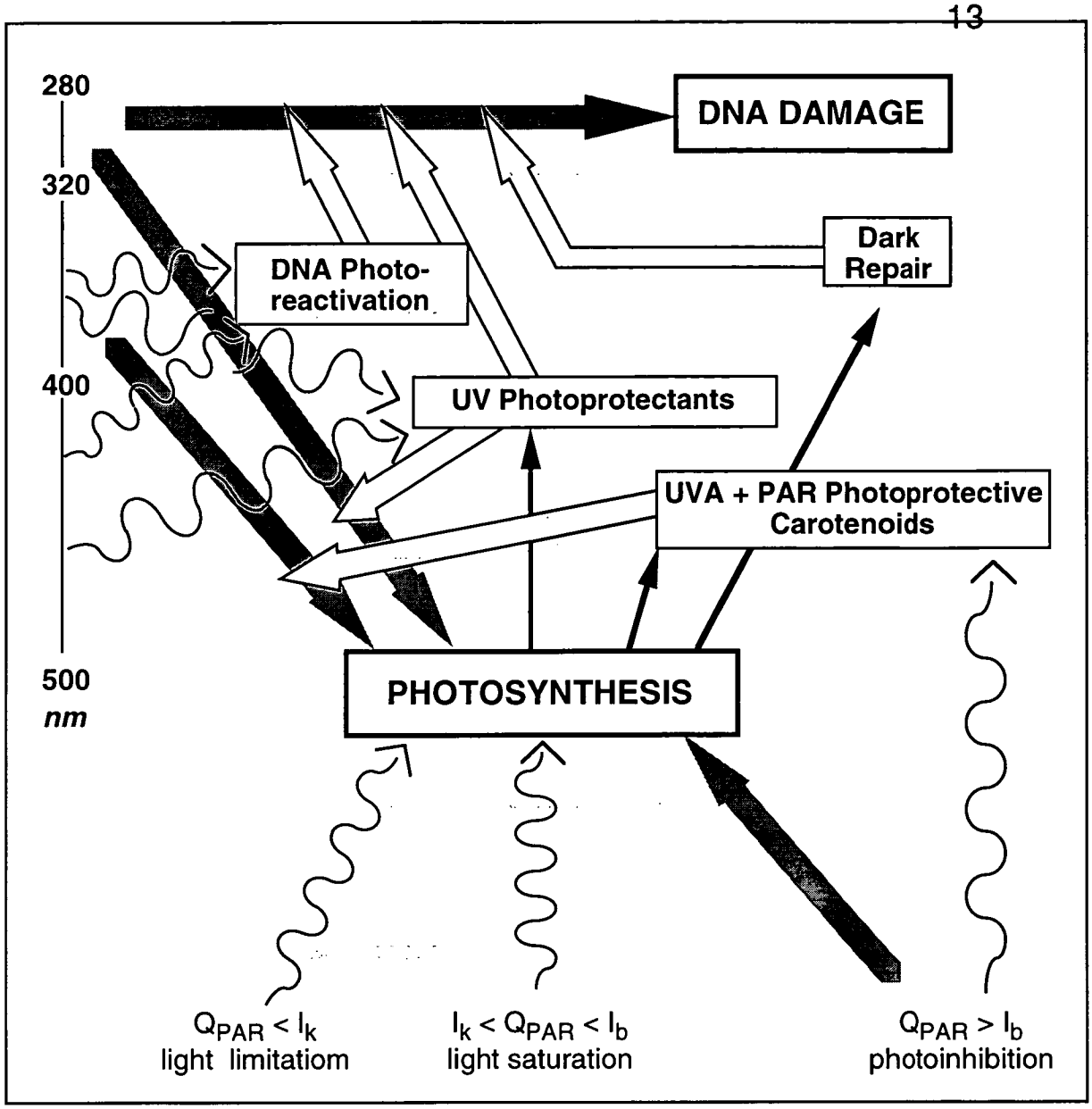
1993, Karentz 1994). Even low UVB irradiance can elicit mutagenic and lethal responses in some organisms (Karentz 1994) and any increase in UVB irradiances is likely to have deleterious effects on plants and animals (eg. Harm 1980, Tevini & Teramura 1989, Karentz 1991).

5. UV TOLERANCE MECHANISMS

Oxygenation of the Earth's atmosphere, and the subsequent development of the ozone layer, occurred over 1 billion years (Fischer 1965, Cloud 1968). Consequently, the first life form probably developed mechanisms of protection and repair to tolerate a hostile UV environment (Yentsch & Yentsch 1982, Karentz 1994). The widespread geographic and taxonomic occurrence of UV protective mechanisms across many forms of life may reflect this ancestral requirement (Karentz 1994). Thus, marine organisms are not defenceless against UVB; instead they possess a diverse capacity to sustain and repair UV-induced damage (Karentz et al. 1991a) (Fig 4). Four mechanisms have been identified that protect organisms against UV damage.

5.1. Avoidance

The most simple means of protecting against UV exposure is selection of a protected environment (under rocks, deep in the water column etc.) (Vincent & Roy 1993, Karentz 1994). However, there are costs for photosynthetic organisms is such habitat selection as they commonly have low total light (Vincent & Roy 1993). The UV intensity and duration experienced by planktonic organisms is determined by the mixed depth and turn-over time. Their only means of avoiding UV exposure is by morphological adaptation of structure including size, shape and outer covering (Karentz 1994) or alteration of buoyancy (Häder 1993). Such adaptations could reduce the surface area to volume ratio, surface area illuminated, surface reflectance and attenuation, and ambient light climate respectively.



Known direct photoinhibitory effects on phytoplankton vitality by high fluxes of Q_{uvb}, Q_{uva} and Q_{par}

Known processes which diminish the photoinhibitory effects of Q_{uvb}, Q_{uva} and Q_{par}

Known light mediated processes which regulate biosynthetic rates

Known biosynthetic processes which regulate photoprotective processes

Fig. 4. A working model of some of the polychromatic and photoregulatory interactions of Q_{uvb} (280-320 nm), Q_{uva} (320-400 nm) and Q_{par} (400-700 nm) on primary production and DNA integrity in phytoplankton. I_k is the minimum Q_{par} requirement to saturate rates of photosynthesis; I_b is the minimum Q_{par} light requirement to photoinhibit rates of photosynthesis (after Prézelin et al. 1993).

Many marine organisms, including phytoplankton, are capable of migration (Burns & Rosa 1980, Häder 1993, Vincent & Roy 1993, Karentz 1994). Vertical migration in the water column is, however, most likely driven by factors other than UV irradiance (Vincent & Roy 1993). In addition, no flagellate algal species has been found that exhibits positive or negative phototaxis solely in response to UV wavelengths (Häder & Worrest 1991). Phototaxic responses to light wavelengths other than UVB would not detect an increase in irradiance only at UVB wavelengths as a result of ozone depletion. UV irradiation also impairs motility (Häder 1993 and refs therein) which would prolong residence time in high UV environments (Vincent & Roy 1993).

5.2. Screening

Considerable research has been devoted to the identification and quantitation of UV-absorbing compounds in a range of organisms from various marine environments. Mycosporine-like amino acids (MAAs) occur in corals (Dunlap & Chalker 1986, Dunlap et al. 1986, 1988), starfish (Nakamura et al. 1981), ascidians (Kobayashi et al. 1981), zoanthids (Ito & Hirata 1977), brine shrimp (Grant et al. 1985), mussels (Chioccara et al. 1979), fish (Chioccara et al. 1980, Dunlap et al. 1989), macrophytes (Tsujino et al. 1980, Wood 1989, Karentz et al. 1991b), cyanobacteria (Scherer et al. 1988) and phytoplankton (eg. Carreto et al. 1990, Marchant et al 1991, Karentz et al. 1991c, Silvalingham et al. 1974). Circumstantial evidence suggests that they fulfil a UV-protective role. UV absorbing compounds occur in organisms occupying habitats which receive high UV and many organisms possessing UV-absorbing compounds are able to increase production of these compounds in response to increased UV exposure (Dunlap et al. 1986, 1989, Caldwell 1981, Scherer et al. 1988, Vernet et al. 1989, 1994, Wood 1989, Carreto et al. 1990, Gieskes & Kraay 1990, Karentz et al. 1991b, Marchant et al. 1991, Shick et al. 1995). Silvalingham & Nisizawa (1990) reported a 1.5 to 28.3 - fold increase in the concentrations of these compounds in the Cyanophyta, Rhodophyta, Phaeophyta and Chlorophyta collected from between 0.5 and 1 m depth between 1975

and 1989. Such large increases suggest an exponential increase in UV-screening as a result of increased UVB exposure. Thus, it is widely accepted that UV-absorbing compounds provide a protective screen to shield cells from the full impact of ambient UV exposure and may constitute a significant protective strategy against UV damage (Caldwell 1981, Dunlap et al. 1986, 1989, Carreto et al. 1990, Karentz et al. 1991b, Vernet et al. 1994) (Fig. 4). Fluorescence emissions by some of these compounds occur at wavelengths that can be absorbed by chlorophyll (Kawaguti 1969, Silvalingham et al 1976). High-energy UV photons may thus be harnessed and substantial energy transferred to the photosynthetic system to benefit rather than damage the cells. However, few studies address the extent to which UV-absorbing compounds benefit the organisms that possess them. There is also little direct experimental evidence that these compounds are primarily UV-absorbers and do not have some other biological functions (Karentz 1994). If they are essential in cellular metabolism, absorption at UV wavelengths may make them a target for UV damage instead of a protective mechanism.

A survey by Karentz et al. (1991c) of some 57 largely subtidal or intertidal species of Antarctic fish, invertebrates and algae found that nearly 90% of those examined contained MAAs. Substantial levels of UV absorbing substances have also been reported in the water containing natural assemblages of Antarctic plankton (Mitchell et al. 1989, Vernet et al. 1989, Gieskes & Kraay 1990). However the number and identity of species possessing such compounds and their effectiveness as a UV screen remains unknown.

5.3. Quenching

UV radiation interacts with various intracellular compounds to produce toxic photoproducts that may be more injurious to the cells than the UV exposure itself. However, marine photosynthetic organisms can reduce damage to the photosynthetic apparatus and photoinhibition caused by UV radiation. Carotenoids can act as photoprotective pigments (Vincent & Roy 1993) (Fig. 4). Such carotenoids as β-carotene, which are involved in photosynthetic electron transfer, can act as radical-

trapping antioxidants (Burton & Ingold 1984), neutralising singlet state oxygen and protecting the photosystem II reaction centre against photooxidation (Jialal et al. 1991, Tefler et al. 1991). Carotenoids also quench triplet state oxygen and may participate in transference of energy to the xanthophyll cycle; functions which dissipate excess energy and protect photosynthetic metabolism (Moore et al. 1982, Demmig et al. 1987). Many marine photosynthetic organisms also have enzymes, such as superoxide dismutase and peroxidase, that scavenge UV-induced oxidants. Any increases in oxygen tension within the cell characteristically causes an increase in concentration of the scavenging enzymes (Shibata et al. 1991, Vincent & Roy 1993).

5.4. Repair

Repair processes are the last line of defence against UV exposure. UV-induced damage to both photosynthetic and genetic material may be repaired (Häder 1993, Tevini 1993, Vincent & Roy 1993, Karentz 1994). DNA can be repaired by four means (see below) but as yet little is known about their relative contribution to DNA repair (Vincent & Roy 1993). Karentz et al. (1991c) reported that the concentration of UVB-induced DNA photoproducts varied 100-fold over 12 Antarctic species of phytoplankton. Thus, enormous interspecific differences exist in sensitivity to DNA damage.

- Photoreactivation or "light repair" occurs when DNA-photolyase is activated by long wavelength UV and short wavelength visible light (310 - 480 nm) (Fig. 4). (Tevini 1993, Karentz 1994). This enzyme recognises and monomerises UV-induced cyclobutyl dimers. This mechanism seems to be a primary means of repairing UV damage in Antarctic phytoplankton (Karentz 1988, 1989, Karentz et al. 1991b). It is, however, dependent on initiating repair immediately after UV damage is sustained (Tevini 1993). Furthermore, the increased ratio of UVB: UVA and PAR as a result of ozone depletion may alter the equilibrium between UVB damage and photoreactivation by increasing the extent of UV-induced damage without increasing the wavelengths that initiate repair (Vincent & Roy 1993).

- Excision or "dark" repair (Fig. 4) requires a number of enzymes to remove a damaged portion of the DNA strand and resynthesise the excised portion using the undamaged DNA strand (Vincent & Roy 1993, Karentz 1994).
- Post-replication repair is a complex process that corrects DNA defects, again using the undamaged DNA strand, but after DNA replication (Karentz 1994).

- UV radiation can cause irrevocable damage to the Photosystem II reaction centre. The photoinhibition repair cycle, over time scales of hours, can resynthesise damaged reaction centres (Andersson et al. 1992).

The effectiveness of the UVB tolerance mechanisms of marine organisms contributes to determining their sensitivity to UVB exposure. However, species differ greatly in their sensitivity to UVB (eg. Calkins & Thordardottir 1980, Joikel & York 1984, Ekelund 1989, Karentz et al. 1991c, Helbling et al. 1992, Smith et al. 1992, Karentz 1994, Davidson et al. in press). Furthermore, the sensitivity of a particular species is not simply a function of the biologically weighted UVB dose, but also depends on dose rate and past light climate (Cullen & Lesser 1991, Helbling et al. 1992, Lesser et al. 1994). Those marine organisms examined, while susceptible to UV induced damage, appear to be incapable of detecting UVB irradiance (Damkaer & Dey 1983, Ekelund 1990, Häder & Worrest 1991, Häder 1993). Given that the ratio of UVB: UVA and PAR has increased up to 2-fold in Antarctica as a result of ozone depletion (see section 3), tolerance mechanisms initiated by UVA and/or PAR irradiance (See Fig 4) may no longer elicit sufficient response to protect cells against UVB damage.

6. RESPONSES OF MARINE ORGANISMS TO UVB RADIATION

Highest UVB irradiances occur in the tropics where solar zenith angles are high and ozone is approximately 50% less than at the poles. Thus, it has been proposed that ozone depletion may have particularly significant ecological consequences in polar latitudes where organisms have evolved under a UV environment that is less in both intensity and

spectral range than those at lower latitudes (Calkins & Thordardottir 1980, Vincent & Roy 1993). That these wavelengths are known to be damaging to a range of organisms has lead to a surge of scientific interest to determine the UV climate of Antarctica and the impacts of UV irradiation on Antarctic organisms (for reviews see Calkins 1982, Worrest 1983, El-Sayed 1988, Hardy & Gucinski 1989, Smith 1989, Smith & Baker 1989, Voytek 1989, 1990, Karentz 1990, 1991, Häder & Worrest 1991, Häder 1993, Tevini 1993 & refs therein, Vincent & Roy 1993, Marchant 1994, Karentz 1994).

6.1 Phytoplankton

Photosynthetic organisms on Earth fix around 200 gigatons of carbon per annum ($GtCyr^{-1}$). Phytoplankton are responsible for an estimated 104 $GtCyr^{-1}$, around half of this photosynthetic production (Houghton & Woodwell 1989). The oceans have a net carbon uptake estimated at around 2.0 ± 0.8 $GtCyr^{-1}$ (IPCC 1994) and constitute a large sink for atmospheric CO_2 (Fig. 5). Phytoplankton photosynthesis facilitates this carbon sink. A significant proportion of phytoplankton cells, and the faeces, moults and organisms that depend on phytoplankton as their source of carbon (Buck et al. 1990, Nicol & Stolp 1989, Nötig & von Bodungen 1989), sinks to intermediate and deep water. As a results, an estimated 20% of the carbon phytoplankton sequester in the surface ocean (IPCC 1994) is lost to the deep oceanic carbon reservoir for geological time-scales (Fig. 5). However, most phytoplankton lack the epidermal UV protection against UV of higher plants and animals (Worrest & Häder 1989) and are physiologically adapted to the subdued light climates that characterise the marine habitat (Vincent & Roy 1993). Because of the high productivity of these organisms, and the role they play in transporting carbon to deep water, even a small decrease in their productivity may have dramatic effects on organisms and carbon cycle processes that depend upon them (Häder 1993).

Proposed scenarios concerning the future for Antarctic phytoplankton range widely. El-Sayed et al. (1990) concluded that Antarctic phytoplankton are UV stressed at present

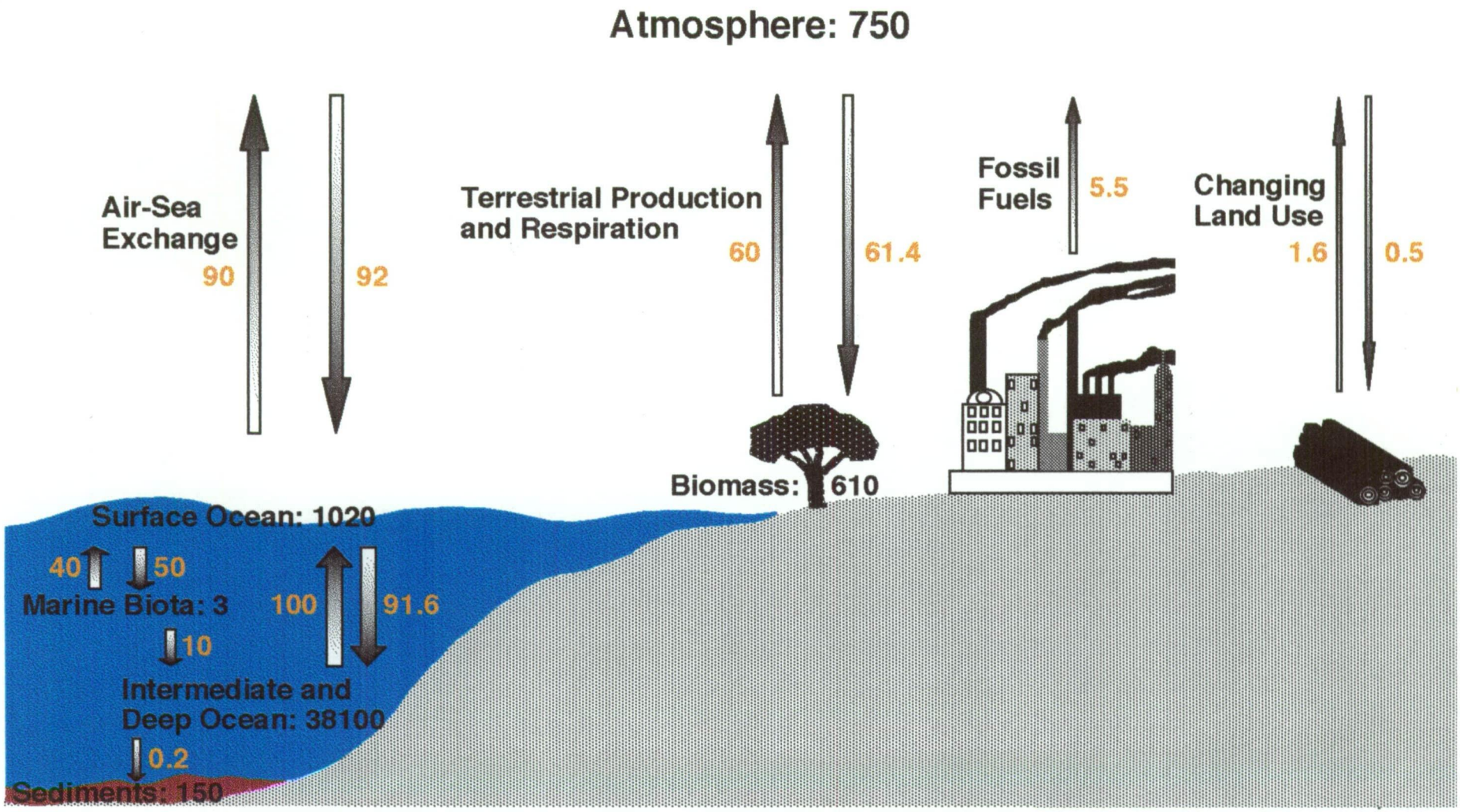


Fig. 5. Global carbon cycle in terrestrial and aquatic ecosystems. Figures in black indicate the size of important carbon reservoirs (GtC), those in gold indicate the magnitude of carbon fluxes ($GtCyr^{-1}$) (after Houghton & Woodwell 1989, IPCC Report 1994).

and are likely to be seriously affected by any increase in UV radiation. In Antarctic waters Holm-Hansen et al. (1989) and Cullen and Lesser (1991) suggest that significant impacts would only be felt in the upper water column and that vertical mixing should ameliorate the impact of UV on phytoplankton.

Phytoplankton form the base of the Antarctic food web and sustain the wealth of life for which the Southern Ocean is renown (Ainley et al., 1986). Light limits productivity in Antarctic waters during winter when sun angles are low and the sea is ice and snow covered (Marchant 1994). The increasing light in spring generates ice algal blooms. In the marginal ice zone (MIZ), release of freshwater from melting ice produces a high light, high nutrient, shallow mixed zone which supports spectacular phytoplankton blooms (Smith 1987). Phytoplankton blooms in the marginal ice zone (MIZ) contribute between 25 and 67% of the phytoplanktonic production in the Southern Ocean (Smith & Nelson, 1986) while sea ice algae contribute 10 - 50% of the primary production in some areas during spring (see Voytek 1989). However, both habitats appear susceptible to UVB radiation as blooms in the ice and MIZ coincide with elevated UVB irradiances resulting from ozone depletion (Fig. 6). Sea ice may be sufficiently transparent to UV that biologically significant doses are received by the ice algal community (Trodahl & Buckley 1989). In the MIZ, mixed depths may be 20 m or less for up to 6 days (Mitchell & Holm-Hansen 1991, Veth 1991) while UVB may penetrate to depths in excess 60 m in Antarctic waters (Gieskes & Kraay 1990, Smith et al. 1992) and reportedly reduces survival in the upper 10m (Karentz, 1989) and photosynthesis in the upper 10 - 25 m (Holm-Hansen et al. 1989, Smith et al. 1992).

6.1.1 UVB and Phytoplankton Production

Much of the research on the responses of phytoplankton to UVB has relied on measurement of carbon assimilation rates. PAR, UVA and UVB have all been shown to elicit photoinhibition in phytoplankton (Jones & Kok 1966, Neale et al. 1994). High PAR irradiances are inhibitory to photosynthesis (Bühlmann et al. 1987) but this is

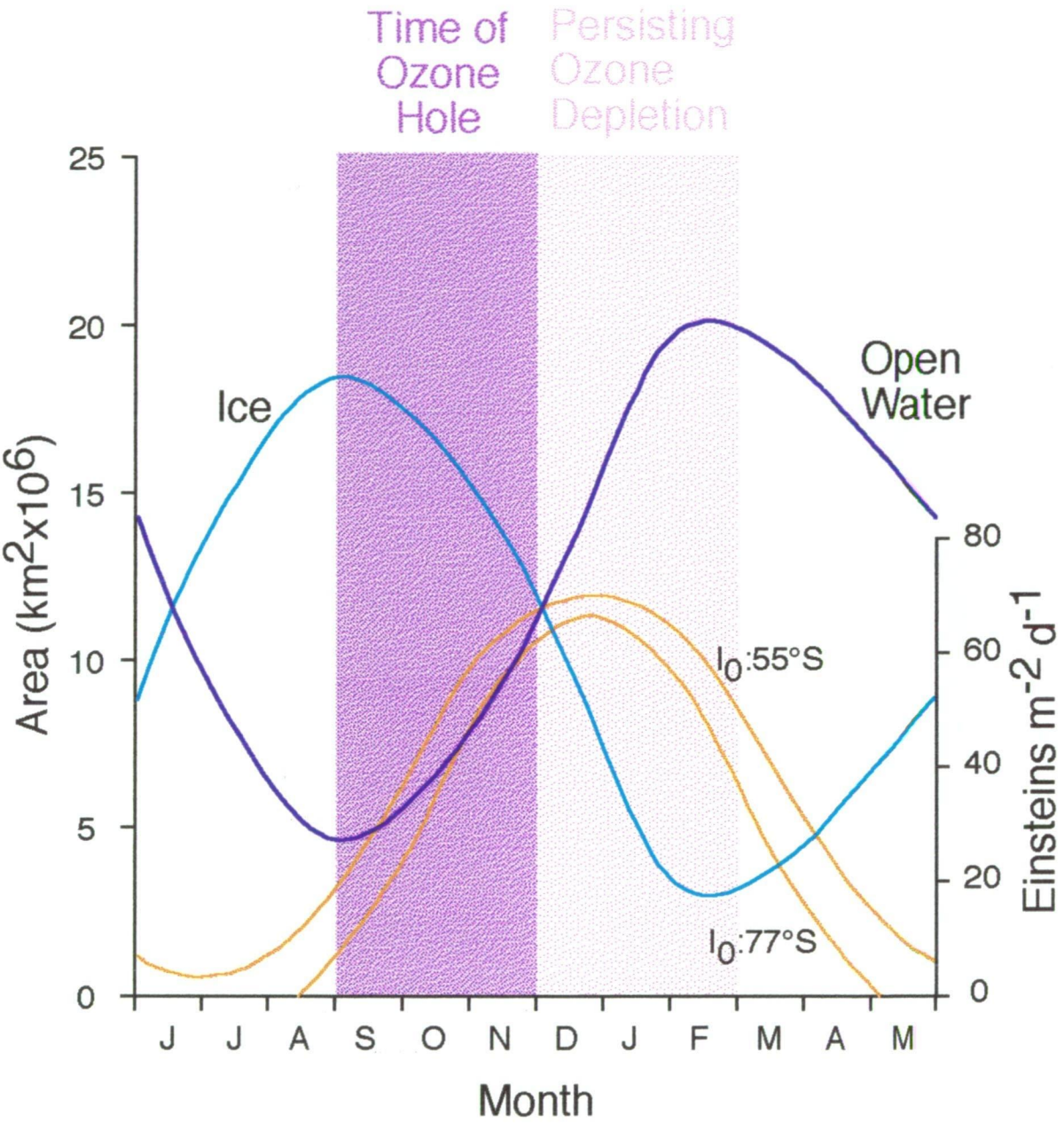


Fig. 6. Annual changes in the area of the Southern Ocean as open water and covered by sea ice and changes in seasonal incident radiation (PAR) at two latitudes. The stippled area indicates the time duration of the ozone hole and persisting ozone depletion (adapted from Helbling et al. 1994).

generally slight (Joikel & York 1984). UV is widely reported as being inhibitory to photosynthesis (Jitts et al. 1976, Lorenzen 1979, Worrest et al. 1981, Jokiel & York 1984, Smith & Baker 1989, Voytek 1989, Häder & Worrest 1991a, Smith et al. 1992, Prézelin et al. 1993, Helbling et al. 1994). Photosynthesis is more severely inhibited by UVB than UVA (Cullen et al. 1992). However, it is UVA that is reportedly responsible for between 50 and 75% of the inhibition of photosynthesis and growth (Jokiel & York 1984, Maske 1984, Holm-Hansen et al. 1989b) as it penetrates to greater depths. Estimates of inhibition by near surface UVB irradiances range from 15 - 60% (Worrest 1983, Maske 1984, Holm-Hansen et al. 1989b, Helbling et al. 1992, Smith et al. 1992) but this inhibitory effect is only experienced in the upper 10 - 25 m (Holm-Hansen et al. 1989b, Smith et al. 1992). In contrast, studies by Gala and Giesy (1991) and Hobson and Hartley (1983) found little inhibition of production by UVB. Their results did show marked seasonal variation in the photoinhibition, with minimum inhibition during summer. This they attributed to increased tolerance of the phytoplankton communities to UVB exposure as a result of changes in species composition and photoadaptation.

Estimates of the depth-integrated decline in primary production as a result of ozone depletion vary. A decline in primary production of 10% and 6 - 12% in the marginal ice zone (MIZ) has been proposed by Voytek (1989) and Smith et al. (1992) respectively and Behrenfeld et al. (1993) and Cullen et al. (1992) estimate a decline of 12 - 15% in near surface Antarctic waters. Helbling et al. (1994) obtained a value of 6.4% reduction in the upper 20 m of the water column during extreme ozone depletion. However, when changes in the magnitude of ozone depletion and the spatial and temporal variations in phytoplankton production were taken into account, Helbling et al. (1994) calculated the loss of primary production over the Southern Ocean as a result of elevated UVB would amount to only 0.15% over a year. In contrast, the change in global production as a result of increased UVB in Antarctic waters estimated by Voytek (1990) was 2 - 4.5%. Thus, despite being the focus of most of the research effort since the discovery of ozone

depletion, controversy surrounds the impact of increased UVB on Antarctic phytoplankton production.

Some evidence suggests changes in the species composition of ice-algae since the appearance of the ozone hole (McMinn et al. 1994). Schofield et al. (1995) measured the photoinhibition of ice algal communities exposed to Antarctic solar radiation beneath ice that was forming at Palmer Station during October. They found UVB-induced photoinhibition increased from 4 - 23% over the day. Furthermore, the quantum yield from photosystem II did not recover from photoinhibition for a further 6 hours after sunset. Ryan and Beaglehole (1994) observed a 5% reduction in ice-algal primary production as a result of exposure to in situ UVB but found that low-level artificial enhancement of UVB wavelengths had no significant effect on primary production or chlorophyll a content / cell. Consequently, they concluded that the effect of ozone depletion on this community was minimal.

6.1.2 UVB, Growth, Survival and Phytoplankton Species Composition

Exposure of phytoplankton to UVB has been shown to reduce photosynthesis (see above) amino acid, protein, ATP, nitrogenase activity and nitrogen metabolism (Döhler 1984, 1987, 1992, Döhler et al. 1987, 1991, Vosjan et al. 1990, Karentz et al. 1991a), and polyunsaturated fatty acid concentrations (Goes et al. 1994, Wang & Chai 1994, Döhler & Biermann 1994). The decline in these essential cell metabolites and processes, together with decreased enzyme activity (Döhler et al. 1991), reduces synthesis of protein, cell membranes, membrane permeability and nutrient assimilation (Claustre et al. 1989, Döhler 1992, Goes et al. 1994, Lesser et al. 1994) and influence the growth, cell division, motility, phototaxis, survival, cell size, life cycle and species composition of phytoplankton communities (Lorenzen 1979, Calkins & Thordardottir 1980, Worrest et al. 1981, Worrest 1983, Döhler 1984, Jokiel & York, 1984, Maske 1984, Häder 1986, Claustre et al. 1989, Hardy & Gucinski 1989, Holm-Hansen et al. 1989b, Ekelund

1990, Häder & Worrest 1991, Smith & Baker 1989, Behrenfeld et al. 1992, Helbling et al. 1992, Sebastian et al. 1994) (Fig. 7).

Most research examining the responses of Antarctic phytoplankton to UV have been conducted using exposures of less than 1 day (eg. Lorenzen 1979, Smith et al. 1980, Worrest et al. 1980, Maske 1984, Cullen et al. 1992, Smith et al. 1992, Holm-Hansen et al. 1993). Few studies examine the longer-term effects of exposure to UVB which necessarily include the effects of photoadaptation, photorepair and changes in species composition (Karentz et al. 1991a, Villafañe et al. 1995) that mitigate the impacts of UVB exposure.

Results of such long-term incubations (days or weeks) indicate that chlorophyll a concentrations, cell concentrations and carbon biomass in UV exposed treatments do not differ significantly from those receiving no UV radiation (Karentz 1994, Davidson et al. 1994, Villafañe et al. 1995). Growth rates of some phytoplankton species were stimulated by UVB radiation (Döhler 1984, Karentz 1994, Davidson et al. in press) and changes in species composition, favouring UV resistant phytoplankton (eg. Häder & Worrest 1991, Karentz 1991, Marchant & Davidson 1991, Vincent & Roy 1993, Davidson et al. in press), can maintain the overall growth and production by phytoplankton communities (Helbling et al. 1992, Karentz 1994, Davidson et al. 1996, Villafañe et al. 1995). If photoadaptive responses are more rapid than in situ changes in UVB irradiance, natural phytoplankton communities can physiologically adapt. Photoadaptation of phytoplankton has been observed over time scales of less than a day. Photosynthesis was less susceptible to UVA and UVB photoinhibition during the middle of the day. In addition, short-term carbon incorporation rates were less than those for all-day incubations (Prézelin et al. 1993). Thus, there is a growing recognition that the results of short-term incubations cannot predict the long-term effects of UVB exposure (Helbling et al. 1992, Behrenfeld et al. 1993). Other long-term studies showed decreased species diversity and interspecific differences in sensitivity but also showed decreased

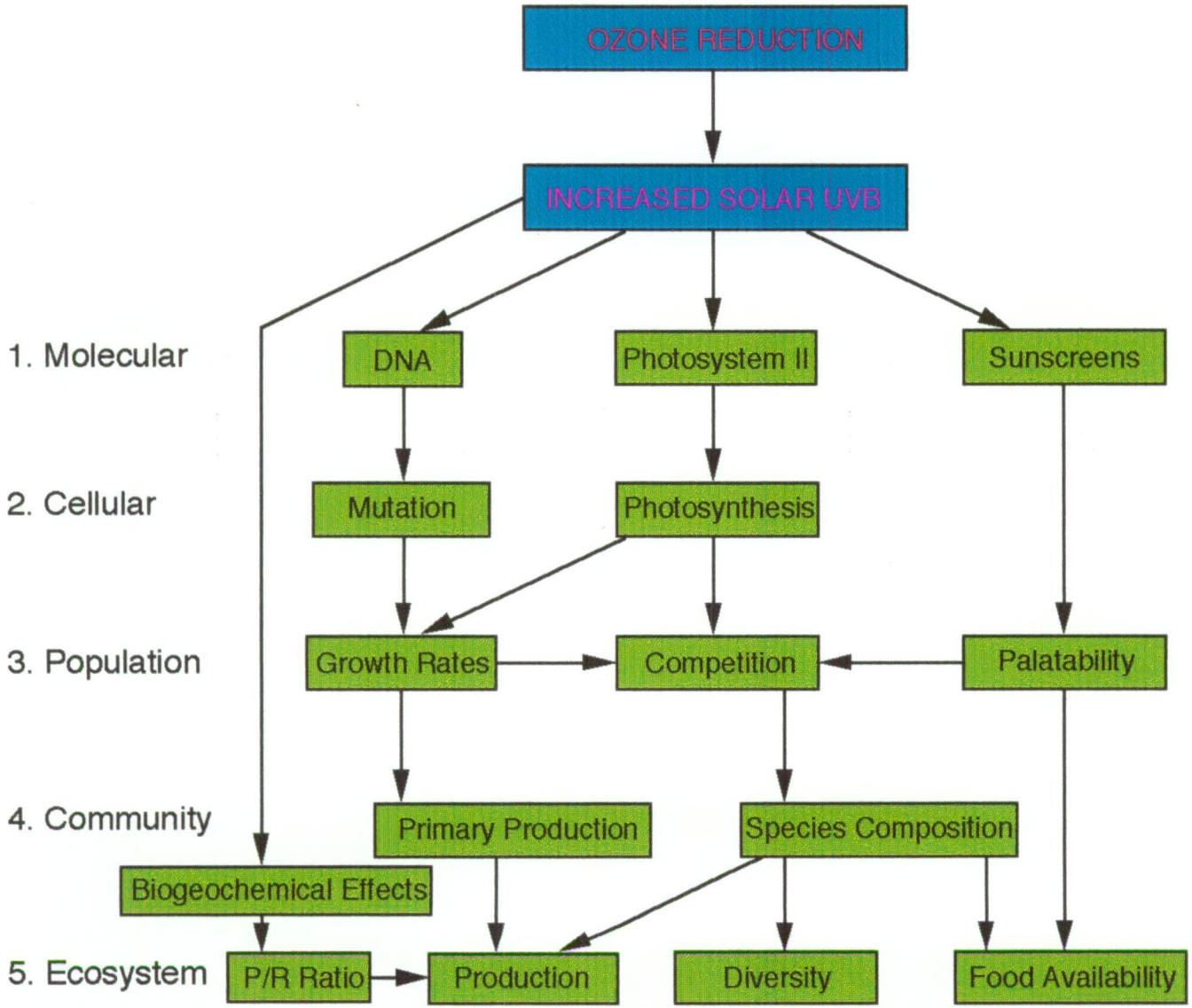


Fig. 7. The pervasive influence of UVB on processes in aquatic photosynthetic organisms from the molecular to community levels (modified from Vincent & Roy 1993).

pigment complement and rates of photosynthesis, growth and biomass production (Worrest et al. 1978, 1981a, 1981b, Thomson et al. 1980, El-Sayed et al. 1990, Behrenfeld et al. 1992).

Species and life cycle stages differ in their growth and survival response to UVB irradiance (Calkins & Thordardottir 1980, Karentz et al. 1991a, Marchant et al. 1991). Karentz et al. (1991a) claims that small phytoplankton species are more susceptible to UVB damage than large-celled species. Bothwell et al. (1993) also found large species became dominant in communities exposed to higher UVB levels and Helbling et al. (1994) found the flagellates exhibited the most severe inhibition of photosynthesis by UVB while diatoms were least sensitive. Nano- and picoplankton are responsible for most of the primary production in Antarctic waters (eg. El-Sayed 1988, von Bröckel 1981, Marchant 1993) and may be at greatest risk from increasing UVB fluxes. My studies (Davidson et al. 1994) have shown no significant correlation between size, shape and UV susceptibility. Nor does possession of a frustule by diatoms appear to be responsible for significant UV attenuation (see Chapter 6).

To obtain ecologically pertinent information regarding potential changes in natural phytoplankton communities as a result of ozone depletion it is crucial that the experimental irradiances contain the wavelength structure, flux rates and time scales likely to be encountered in the natural environment. The period for which phytoplankton are exposed to a given UV irradiance clearly alters the nature and magnitude of their response (see section 6.1.1 & 6.1.2). Short-term exposures (0.5 - 4 hours) (eg. Lorenzen 1979, Worrest et al. 1980, Maske 1984) are instructional in determining the acute response of organisms but provide little information regarding mitigation of UVB impacts as a result of photoadaptation. Such studies are, therefore, likely to over-estimate the effect of UVB on natural phytoplankton assemblages. Conversely, studies examining the responses of planktonic organisms to high UVB fluxes during long-term incubations (conducted over weeks or months) (eg. Joikel & York 1984, El-Sayed 1988, Karentz

1994, Villafañe et al. 1995) are of questionable value as marine phytoplankton will never experience such exposures. Thus, much of the uncertainty regarding the impact of increased UVB on marine phytoplankton appears to be due to the time scales and experimental conditions under which the estimates are made (Vincent & Roy 1993).

6.2 Impacts of UVB on Other Marine Organisms

While the impacts of UVB on phytoplankton have received extensive attention, its effect on other organisms has largely been neglected. Organisms such as bacteria, microheterotrophs, crustaceans and fish play vital ecological, commercial and biogeochemical roles in marine waters. Yet little is known about their susceptibility to UVB damage. Furthermore, the impact of UVB-induced changes in their populations on the marine biota is the subject of considerable conjecture (eg. Häder 1993, Marchant 1994) but little evidence.

Herndl et al. (1993) showed UVB radiation reduces bacterioplankton activity by approximately 40% in the upper 5 m in temperate coastal waters. It also reduced bacterial thymidine and leucine incorporation, cell production, cellular enzyme activity to a depth of 10 m in oligotrophic waters. In addition, UVB photochemically degraded bacterial enzymes, thereby reducing enzymatic breakdown of organic substrates (Herndl et al. 1993). Exposure of natural bacterial assemblages near-surface ambient UV radiation in Antarctic surface waters greatly reduced their survival but 81% of the UV-induced mortality was due to UVA wavelengths and there was no significant UV-induced inhibition of bacterioplankton below 9.5 m (Helbling et al. 1995). Similarly, survival of bacterial spores and faecal coliforms is greatly reduced by exposure to natural Antarctic UVB irradiance (Puskeppeleit et al. 1992, Statham & McMeekin 1994). Bacterioplankton also possess UVA-activated DNA repair mechanisms which facilitated recovery after UVB damage (Helbling et al. 1995, Müller-Niklas et al. 1995). Garabetian (1991) found no significant inhibition of bacteria in near surface incubations conducted in the Mediterranean Sea but did propose photoadaptation of the near-surface community to the

high UV environment. Viral mortality also reportedly increases as a result of UVB (Murray & Jackson 1993) and may reduce virus-induced bacterial mortality. The extent to which bacterioplankton survival and metabolism will be reduced by increased UVB remains unknown. Furthermore, nothing is known about the effects of increased UVB on microheterotrophs despite their important role in carbon dynamics (Marchant 1994). Information on the effects of UVB on bacteria, viruses and microheterotrophs is urgently required.

UVB is also damaging to larval fish (Hunter et al. 1981) and larval and adult crustaceans including shrimps, crabs and euphausiids (Hardy & Gucinski 1989, Damkaer & Dey 1983). The susceptibility of marine invertebrates to UVB exposure varied greatly (Dey et al. 1988) but the bulk of these organisms have eggs and larvae that are habitually at or near the sea's surface where they would be exposed to increased levels of UVB as a result of ozone depletion (Hardy 1982). In Antarctic waters, krill swarm close to the surface at the continental shelf and in the MIZ, while benthic fish have a pelagic juvenile stage (Higginbottom & Hosie 1989). Depletion of stratospheric ozone has changed both the magnitude and timing of UVB irradiance, both of which change the susceptibility of these organisms to UVB radiation (Damkaer & Dey 1983). Fish and crustaceans also use UVA or visible light in photoreactivation but are incapable of detecting UVB. Damkaer and Dey (1983) show that they are attracted to light and are eventually killed by UVB light under low UVA and visible light conditions. This may be an important factor given the increased ratio of UVB: UVA and visible light wavelengths (Damkaer & Dey 1983). The extent to which fish, crustaceans and higher trophic levels, including seals, penguins, will be impacted by increases in UVB are currently unknown. Seals and penguins have body protection in the form of feathers and fur (Karentz 1994, Marchant 1994) while birds have a high threshold for UV-induced corneal damage (Hemmingsen & Douglas 1970). However, Antarctic seals and penguins customarily breed and/or rear their young during the Antarctic spring and summer and concern has been expressed

about the effect of increased UVB on their eye, surrounding tissue, and exposed nasal skin (Karentz 1994, Marchant 1994).

7. IMPACTS OF UVB ON MARINE CHEMISTRY

Any UVB-mediated increase in the inhibition of bacterioplankton in surface waters as a result of ozone depletion would reduce the recycling of nutrients and decomposition of dissolved (DOC) and particulate organic carbon (POC) (Herndl et al. 1993). However, photolysis of DOC to dissolved inorganic carbon (DIC) by UV may increase in surface waters if UVB fluxes increase (Zepp et al. 1995). DOC is one of the largest reservoirs of carbon on earth and photochemical degradation of refectory DOC is a rate limiting step in the removal of a large fraction of oceanic DOC (Kieber et al. 1989, Mopper et al. 1991). UVB is also involved in the photolysis of colloidal iron, increasing the availability of this essential, and potentially limiting, micronutrient to phytoplankton (Wells et al. 1991). Thus, changes to bacterioplankton activity and photolysis as a result of increased UVB flux may effect the global carbon cycle and phytoplankton dynamics; the extent of these changes and their impact are currently unknown.

8. IMPACT OF UVB ON THE INTERACTIONS BETWEEN PHYTOPLANKTON AND THE BIOTIC AND ABIOTIC ENVIRONMENT

Exposure of marine phytoplankton to UVB elicits a range of responses (see section 6). This review has dealt with these largely in isolation but the pervasive effects of UVB on phytoplankton may influence ecosystem function (Fig 7). Only Bothwell et al. (1994) has inadvertently (Culotta 1994) studied the impact of UVB on the interaction between phytoplankton and grazers. In this study, Bothwell et al. (1994) showed that benthic freshwater diatoms were inhibited by UVB irradiance but that the dominant grazers, larval chironomids, were even more sensitive, and as a consequence, the diatoms proliferated. Thus, UVB-induced changes in trophic interaction can greatly alter the outcome in comparison with studies using single trophic levels. However, the lack of

information regarding the impact of UVB on higher trophic levels means that their role in determining UVB-induced changes in the Antarctic marine community is unknown (see section 6.2). UVB can also reduce phytoplankton production, growth, biomass, species diversity and polyunsaturated fatty acid concentrations (see section 4 & 6.1.2). The sensitivity of phytoplankton is also mediated by abiotic environmental factors such as the salinity (Döhler 1984) and nutrient concentrations (Cullen & Lesser 1991, Döhler 1992, Bothwell et al. 1993, Behrenfeld et al. 1995). The extent to which increased UVB as a result of the ozone depletion will impact upon natural phytoplankton communities is poorly understood and the consequent changes in the quality and quantity of nutrition available to higher trophic levels in Antarctic waters, is little more than speculation (eg. Sargent & Whittle 1981, Watanabe et al. 1983, Claustre et al. 1989, Hardy & Gucinski 1989, Marchant & Davidson 1991, Karentz 1994).

9. CONCLUSION

Evidence abounds that UVB is damaging to Antarctic organisms. Ozone depletion has increased incident UVB radiation in Antarctica since the mid-1970s yet there is no clear evidence of UVB-induced changes in the marine ecosystem (McMinn et al. 1994). UVB is, however, only one of a suite of environmental stresses in the Antarctic ecosystem (Marchant 1994). These organisms thrive in a turbulent medium, they are highly variable in space and time, and detailed quantitative data describing their pre-ozone distribution and abundance is sparse (eg. Häder 1993, Marchant 1994, Smith et al. 1988). In addition, the nature and duration of UV exposure in Antarctica waters is yet to be fully determined. The interaction of in situ UV intensity, dose and the photophysiology of individual species is complex and the impact on the organisms is not great. However, the shallow blooms of the MIZ, which are responsible for much of the primary production in the Southern Ocean, appear vulnerable to increased UVB radiation as a result of stratospheric ozone depletion (Marchant & Davidson 1991). The consequent changes in

phytoplankton species composition may be sufficiently slow or slight that they are undiscernible from spatial and interannual variability.

Karentz (1990) considers that interspecific differences in ability to cope with UV may prove crucial in determining the ecological impact of elevated levels of UVB. Interspecific differences in UV tolerance have been reported (Karentz et al. 1991a, Calkins & Thordardottir 1980, El-Sayed et al. 1990, Karentz 1988, Vosjan et al. 1990, Worrest et al. 1978, 1981a), yet remarkably little in known about the UV photobiology of key species of Antarctic marine phytoplankton. This thesis specifically examines the comparative photophysiology of key Antarctic phytoplankton species, their ecological role in Antarctic waters and the possible ramifications of increased UVB for the Antarctic ecosystem.

CHAPTER 2

Antarctic marine phytoplankton

1. INTRODUCTION

UVB detrimentally effects the growth and production of phytoplankton in Antarctic waters but the impact of UVB exposure is species specific (Chapter 1). These interspecific differences may be crucial in determining the ecological impact of increased levels of UVB on the Antarctic marine biota (Karentz 1990). To appreciate the role of these species and the potential impact of their photobiological responses on the Antarctic ecosystem it is necessary to gain a broader appreciation of phytoplankton in Antarctic waters. There are approximately 330 species of phytoplankton in Antarctic waters (Marchant et al. in prep.). Plainly, studies of the UVB photobiology of phytoplankton should concentrate on key species. The phytoplankton examined in this thesis are species or members of genera that are significant or dominant components of the Antarctic phytoplankton. This, chapter briefly examines the species composition, abundance and ecology of Antarctic phytoplankton, especially in the sea ice and MIZ.

2. CHANGING PARADIGMS

The early expeditions of J.D. Hooker aboard the "Erebus" and "Terror" in 1839 and 1843 led to the belief that the Southern Ocean contained an unrivalled phytoplanktonic richness and that this was composed primarily of large diatoms (eg. Hooker 1847, Hart 1942). This view persisted over the ensuing century and has only been rectified recently (eg. El-Sayed 1970, Fukuchi 1980, Marchant 1993). Antarctic waters can support high standing crops of phytoplankton (Holm-Hansen et al. 1977), but the supposed richness of Antarctic waters, with a few exceptions such as the Weddell Sea (El-Sayed 1984), is only truly representative of coastal and inshore regions rather than ice-free waters (El-Sayed 1970, Hempel 1985). These ice-free waters of the Southern Ocean are, however,

rich in inorganic nutrients, creating the paradox of Antarctic phytoplankton namely, "starving in the midst of plenty" (Priddle 1990). Numerous authors have addressed the reasons for this paradox (Jacques 1983, El-Sayed 1984, Holm-Hansen 1985, Smith & Nelson 1985, Priddle et al. 1986, Billen et al. 1987, Martin et al. 1990).

During winter the principal factor limiting phytoplankton growth at these high latitudes is the absence of light (El-Sayed 1970, 1984). However, the limited annual phytoplanktonic production in oligotrophic regions of the Southern Ocean has been variously attributed to iron availability (Martin et al. 1990), energetic vertical mixing carrying cells beneath their critical depth (Mortan-Bertrand 1988, 1989, Smith & Nelson 1985), low metabolic rate as a result of low ambient temperature (Neori & Holm-Hansen 1982), sinking (Schnack et al. 1984) and grazing by heterotrophs from protozoa to krill (Schnack et al. 1984, Wefer et al. 1988). The relative impact of each of these factors on phytoplankton standing stocks and net production would differ spatially and temporally.

Methods originally used for collection, preservation and handling of phytoplankton were inappropriate for small fragile cells and the persistence of large robust cells gave rise to the view that Antarctic phytoplankton consisted predominantly of large diatoms (Marchant 1993). Conversely, it now appears that high concentrations of chlorophyll a in Antarctic waters are less commonly due to microplanktonic (largest cell dimension of 20–200 μm) diatoms than in temperate and tropical waters (El-Sayed & Weber 1982). Nano- and picoplanktonic phytoplankton (2–20 μm and 0.2–2 μ m respectively) are frequently the greatest source of chlorophyll a (eg. Marchant 1993, Kivi & Kuosa 1994). Identification and appreciation of the role played by photosynthetic nano- and picoplankton was aided since the 1950s by the availability of electron microscopes, the advent of chlorophyll a estimation to estimate phytoplankton biomass (Richards & Thompson 1952), and the ability to estimate primary production using ^{14}C (Steemann Nielsen 1952).

3. SPECIES COMPOSITION AND ABUNDANCE IN THE SOUTHERN OCEAN

While the bulk of the ocean surrounding Antarctica exhibits low chlorophyll a concentrations (eg. Saijo & Kawashima 1964, El-Sayed 1970, Fukuchi 1980), regions of high phytoplankton standing stocks and production occur in inshore waters surrounding Antarctic and Sub-Antarctic islands, and the Antarctic continent, frontal zones such as the Polar Front, and in the pack-ice and marginal ice zone (MIZ) (for review see El-Sayed 1988). Sea ice algae can contribute 10 - 50% of the primary production in some areas during spring (Voytek 1989, Knox 1990) and phytoplankton blooms in MIZ contribute between 25 and 67% of the phytoplanktonic production in the Southern Ocean (Smith & Nelson 1986). The large accumulations of krill, seabirds, penguins, seals and whales associated with the ice and MIZ in spring reflect the major contribution made to the Antarctic food web by phytoplankton from these habitats (Smith et al. 1988). As well as being amongst the most productive waters in the Southern Ocean, phytoplankton in these habitats are also most likely to experience increased ambient UVB (see Chapter 1). Thus, phytoplankton inhabiting these environments will be examined in some detail.

Approximately 330 taxa, including diatoms, dinoflagellates, prasinophytes, cryptophytes, parmales, haptophytes and cyanobacteria, occupy the Southern Ocean; the largest marine ecosystem on the globe (Hedgepeth 1977). Some of these algal taxa are poorly represented in Antarctic waters. Cyanobacteria are essentially absent. Their concentration declines exponentially with decreasing temperature across the Southern Ocean; from around 10^7 cells l^{-1} close to Tasmania, down to around 10^3–10^4 cells l^{-1} in Antarctic coastal waters (Marchant et al. 1987, Letelier & Karl 1989, Rivkin et al. 1989). Coccolithophorids (Haptophyceae) also decrease in concentration southward and are sparse in waters south of 60°S (Jacques & Panouse 1991, Nishida 1986). However, lightly silicified coccolithophorids, recently recognised as being heterotrophic, reach

maximum abundance in polar waters including those surrounding Antarctica (Marchant & Thomsen 1994).

Prasinophytes such as Pyramimonas gelidicola McFadden, Moestrup & Wetherbee may attain high concentrations (McFadden et al. 1982, Bird & Karl 1991, McMinn & Hodgson 1993). Cyptophytes are widespread in Antarctic waters but are only rarely abundant (Buma et al. 1992, Brandini 1993, Jacques & Panouse 1991, Kang & Lee 1995). Anecdotal and published information suggests that concentrations of the cryptophyte Geminigera (formerly Cryptomonas) cryophila (Taylor & Lee) Hill and an undescribed Geminigera sp. have increased during recent years off Davis Station (McMinn & Hodgson 1993, Mura et al. 1995, Gibson et al. submitted) and McMurdo (pers. comm. W. Smith Jr). However, pigment studies indicate that the occurrence of these taxa in Antarctic waters is highly variable in space and time (Bidigare et al. 1986, Buma et al. 1992) and that they are commonly a minor component of the phytoplankton. The Parmales is a recently erected order containing eleven species (Booth & Marchant 1987). These cryptic organisms were first thought to be siliceous cysts or the resting stage of a choanoflagellate (Silver et al. 1980) but are now tentatively placed in the Crysophyceae (Booth & Marchant 1987). They appear to be widespread around Antarctica (Buck & Garrison 1983, Marchant & McEldowney 1986, Takahashi et al. 1986) and occur in highest concentrations (around 2×10^5 cells l^{-1}) in waters less than 2°C and between 75 and 100 m depth (Nishida 1986). Dinoflagellates, which are amongst the most abundant protists in temperate and tropical waters, were regarded as being poorly represented in Antarctic waters (Dodge & Priddle 1987). Recent investigations indicate that they are relatively abundant. Photosynthetic dinoflagellates may reach concentrations around 10^6 cells l^{-1} in brine channels (Stoecker et al. 1992) and heterotrophic members of this division are important grazers (Bjørnsen & Kuparinen 1991, Brandini 1993, McMinn & Hodgson 1993), constituting up to 97% of the protozoan biomass (Lessard & Rivkin 1986).

The use of new and improved methods, and the extended geographic and temporal range of sampling has demonstrated that many taxa contribute significantly to the phytoplankton of the Southern Ocean (Hentschel 1932, Balech & El-Sayed 1965, Hasle 1969, Buck & Garrison 1983). However, numerous studies of protists in sea ice and at ice edges in Antarctica have been conducted to ascertain the species composition and abundance of protists, and these repeatedly demonstrate dominance of the phytoplankton community by diatoms and/or Phaeocystis antarctica Karsten (eg. Hoshiai 1977, Buck & Garrison 1983, McConville & Wetherbee 1983, Garrison et al. 1987, Lipski 1987, Perrin et al. 1987, Fryxell & Kendrick 1988, Garrison & Buck 1989a, Davidson & Marchant 1992a). Thus, P. antarctica and diatoms remain the most abundant primary producers, dominating autotrophic biomass and the species composition in the sea ice and the water column in the Southern Ocean (eg. Bunt & Wood 1986, Vincent 1988, Smith & Nelson 1985, El-Sayed & Fryxell 1993, Scott et al. 1994). Diatoms also dominate the species composition of Antarctic waters as around 200 species inhabit the Southern Ocean (Hasle 1969, Priddle 1990, Marchant et al. in prep).

Garrison et al. (1983) reported a high degree of similarity in species composition between the Weddell Sea ice and the planktonic communities. Such diatom species as Fragilariopsis curta (V.H.) Krieger and F. cylindrus (Grunow) Krieger may dominate the phytoplankton community both in the sea ice and the water column (eg. Burkholder & Mandelli 1965, McConville & Wetherbee 1983, Gersonde 1986, Garrison et al. 1987, Fryxell & Kendrick 1988, Fryxell 1989, Davidson & Marchant 1992a) even during the winter months (Moisan & Fryxell 1993). However, the relative abundance of species frequently differs between these two environments allowing characterization of their diatom communities.

3.1. Sea Ice Algae

At its maximum extent (September) sea ice covers approximately 20 million km^2 of the Southern Ocean and recedes to 4 million km^2 at its minimum in February (Zwally et al

1983). Implicit in these figures is the fact that Antarctic sea ice is predominantly less than one year old and this vast, transient environment is inhabited by organisms from bacteria to mammals and the ice-algal community supports bacteria, protozoa and metazoa (Garrison 1991a, b).

Sea ice encompasses a range of ice types including brash, platelet, frazil, grease, pancake and congelation ice (Squire 1990). These ice types may form pack ice during the onset of winter, which may in turn become rafted or form tide cracks and pressure ridges. These types of ice differ greatly in their structure and formation. Frazil ice nucleates on and harvests particles, including phytoplankton cells, from the underlying water column (Garrison et al. 1983). Accretion of frazil to the under-surface of existing ice flows can contribute much of their growth of floes (Clarke & Ackely 1984). In contrast, congelation (columnar) ice, which forms in waters of low turbulence, tends to exclude particles such as phytoplankton from the ice column as it forms (Clarke & Ackely 1984).

Gradients of light, temperature, salinity and ice melt, together with varying snow cover, light attenuation by the biotic community and the physical structure of the ice, cause marked environment heterogeneity within the ice which is reflected in the spatial heterogeneity of the algal distribution and abundance (eg. Sullivan et al. 1984, Maykut 1985, Horner et al. 1988, Kottmeier & Sullivan 1988, Bartsch & Diekmann 1988). Differences in the physical and chemical environment within and beneath the ice means that algae occupy the sea ice to varying extents with differing rates of growth and survival in each environment (Horner et al. 1988).

Phytoplankton first bloom during spring in the sea ice as this environment receives the maximum light at latitudes south of around 60°S (Squire 1990). However, the distribution of algal assemblages indicates several relatively distinct ice microhabitats (Horner et al. 1988) These include surface layer assemblages on pack or fast ice, interior ice algae in pack ice (trapped by accretion of frazil ice), fast ice blooms in congelation ice (either trapped as it formed or in brine channels), bottom layer assemblages (either in the

lower 20 cm of congelation ice or in platelet ice beneath consolidated floes) and sub-ice assemblages of mat or strand forming algae (Garrison 1991b). The bottom- and sub-ice communities are commonly extensive and productive (McConville & Wetherbee 1983, Palmisano & Sullivan 1983, Hoshiai 1981). Mat-, chain- and tube-forming species that are anchored to the under surface of the ice may also hang down into the seawater beneath (Watanabe 1988).

Approximately 80 species, or around half of the diatoms occurring in Antarctic waters, have been observed in the sea ice (Garrison 1991b). However, the sea ice algal assemblages are commonly dominated by such attached species as Pleurosigma Smith sp., Berkeleya rutilans (Trentepohl ex Roth) Grunow, Entomoneis kjellmanii (Cleve) Poulin & Cardinal, Psuedonitzschia turgiduloides Hasle, Nitzschia stellata Mangin, Cocconeis Ehrenberg spp., Fragilaria islandica var adeliae Grunow (in V.H.) and the Fragilariopsis Hustedt species F. curta, F. cylindrus and F. kerguelensis (O'Meara) Hasle (Bunt & Wood 1986, McConville & Wetherbee 1983, Grossi et al. 1984, Grossi & Sullivan 1985, Everitt & Thomas 1986, Horner et al. 1988, Watanabe 1988, Garrison et al. 1987, Garrison 1991b, Scott et al. 1994). Unattached forms include species of Coscinodicsus asteromphalus Ehrenberg, Asteromphalus Ehrenberg spp. and Navicula glaciei V.H. (eg. Bunt & Wood 1986, Whitaker 1977). Most studies report the pennate members of the Fragilariopsis group as the dominant ice-associated diatoms but centric species such as Stellarima microtrias, Thalassiosira gracilis Karsten and T. antarctica Comber may also be abundant (eg. Watanabe 1982, Garrison et al. 1987). The haptophyte, Phaeocystis antarctica is the only species other than diatoms that is frequently reported as dominating the ice algae, reaching concentrations as high as 5×10^7 cells l^{-1} (eg. Bunt & Wood 1986, Garrison et al. 1987, Fryxell & Kendrick 1988).

The importance of the sea ice community in the Antarctic marine food web remains uncertain (Garrison 1991b). However, it has been shown that krill actively seek out and graze the microbial biomass in the ice community (eg Stretch et al. 1988, Marschall

1988). Protozoa also occur in the sea ice community including most Antarctic choanoflagellates, euglenoids, auto- and heterotrophic dinoflagellate, colourless flagellates such as bodonids, and numerous species of ciliate (Garrison 1991a, b). Sea ice algae may also play another important role in Antarctic waters. The ephemeral nature of this habitat means that phytoplankton are released during the and can act as a "seed" source for ice edge blooms (eg. Garrison et al. 1987, Fryxell & Kendrick 1988).

3.2. The Ice Edge Phytoplankton

As the Antarctic pack ice retreats southward during austral spring it trails a region where fresh water released by the melting ice forms a pycnocline of 20 m depth or less which may persists for up to 6 days (Mitchell & Holm-Hansen 1991, Veth 1991) (Fig. 1) and extend some 200 km seaward of the ice front (Smith & Nelson 1986). Phytoplankton, confined to these shallow mixed depths in the marginal ice zone (MIZ) experience a high light, high nutrient environment. The resulting phytoplankton blooms support high algal biomass in waters surrounding Antarctica (El-Sayed & Taguchi 1981, Nelson & Gordon 1982, Garrison & Van Scoy 1985, Smith & Nelson 1985, Nelson et al. 1985) and contribute 25 to 67% of phytoplanktonic production in the Southern Ocean (Smith & Nelson 1986). This southward moving region of high productivity is coupled to higher trophic levels (Ainley et al. 1986) providing much of the carbon required to sustain the large populations of zooplankton, birds and mammals for which the Southern Ocean is noted (Ross & Quetin 1986, Sakshaug & Skjoldal 1989). Despite their contribution to the food web, much of the ice-edge phytoplanktonic production is not consumed by grazers and apparently sediments from the euphotic zone (Smith & Nelson 1986, von Bodungen et al. 1986, Fischer et al. 1988) to form massive deposits of diatomaceous ooze in Southern Ocean sediments (Truesdale & Kellogg 1979). Turbulent mixing in the

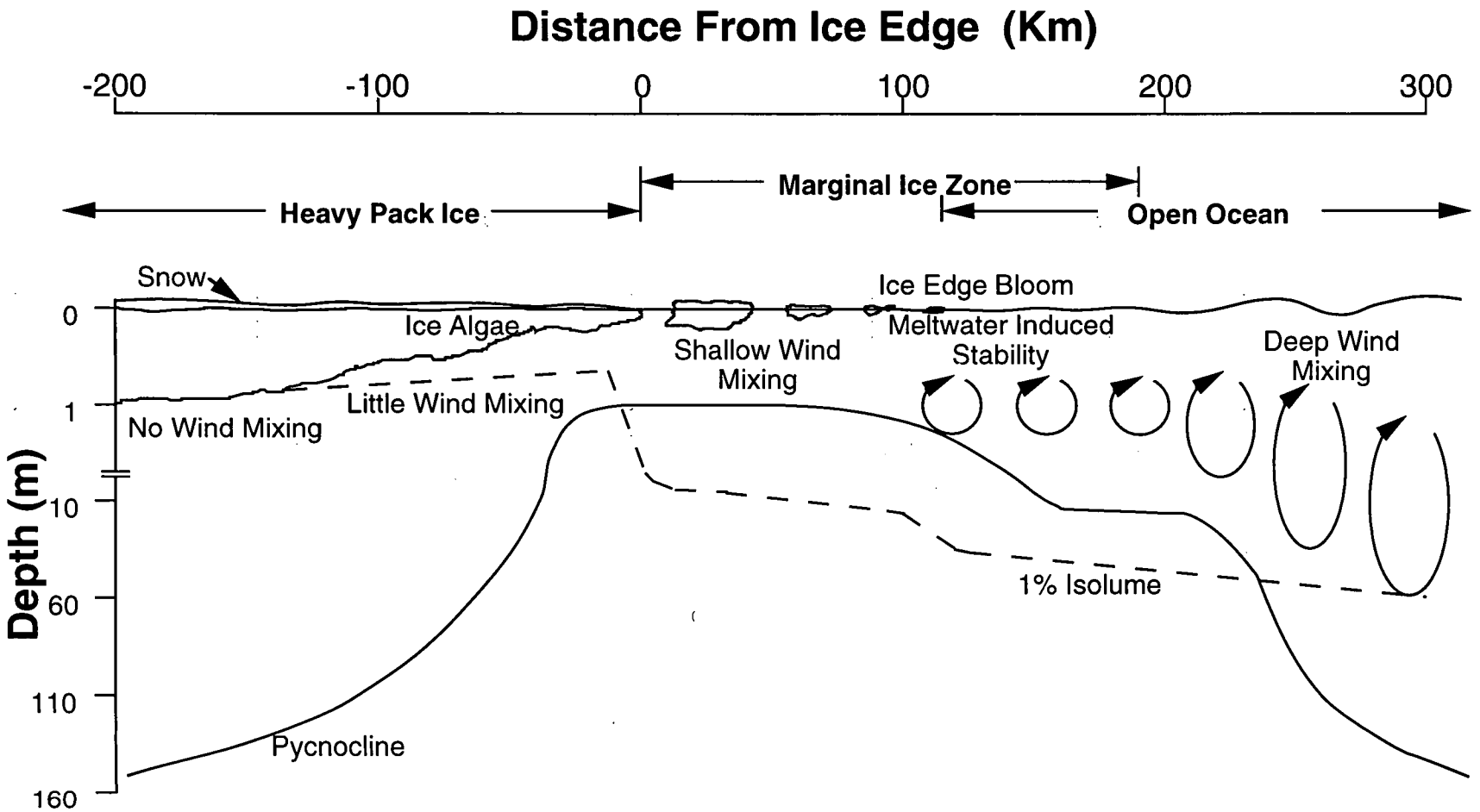


Fig. 1. Schematic model of the environmental conditions at the marginal ice edge that are neccessary for a sustained phytoplankton bloom. Redrawn from Sullivan et al. (1988).

region of the MIZ eventually deepens or disrupts the pycnocline and the ice edge blooms subside (Fig. 1).

The most abundant components of the phytoplankton of the marginal ice edge zone are diatoms and the prymnesiophyte Phaeocystis antarctica (Garrison & Buck 1989b, Garrison et al. 1987, Fryxell & Kendrick 1988, Davidson Marchant 1992b). P. antarctica is often the first alga to bloom, may reach concentration as high as 6×10^7 cells l^{-1} (Davidson & Marchant 1992b) and can alone contribute up to 37% of the annual open water production at some Antarctic sites (Palmisano et al. 1986). The remainder of the annual production is comprised of diatoms, principally of the genera Nitzschia Hassall and Fragilariopsis. Blooms comprised almost entirely of Fragilariopsis curta have been found to extend 200 km seaward of the receding pack ice and sequester as much as 962 $mgCm^{-2}day^{-1}$ (Wilson et al. 1986). However, many other phytoplankton species are reportedly major contributors to and/or dominate the phytoplankton blooms in the MIZ. Perhaps the richest and most extensive of these blooms was that reported by El-Sayed (1971) which was composed almost entirely of Thalassiosira tumida (Jan.) Hasle covered an area of around 15,000 km^2 and attained chlorophyll a up to 190 mg/m^3. Other prevalent diatom species in ice edge blooms include Fragilariopsis nana (Steeman Nielsen) Paasche, Porosira glacialis (Grunow) Jørgensen, Fragilariopsis cylindrus, Cylindrotheca closterium Ehrenberg, Corethron criophilum Castracane, Odontella weissflogii (Jan.) Grunow, Proboscia alata (Brightwell) Sunström, and the Chaetoceros Ehrenberg species C. dichaeta Ehrenberg, C. neglectum Karsten and C. socialis Lauder (eg. Krebs 1983, Theriot & Fryxell 1985, Heywood & Priddle 1987, Kang & Fryxell 1991, 1992, 1993). The prasinophyte Pyramimonas gelidicola and cryptophyte Geminigera spp. may also be contributors to ice-edge blooms (McFadden et al. 1982, Hewes et al. 1985, Bird & Karl 1991, McMinn & Hodgson 1993, Gibson et al. in prep). High concentrations of Gymnodinium sp. have also been observed in brine channels and the surface-ice community (Stoecker et al 1992). Here again, heterotrophic nanoflagellates, dinoflagellates, choanoflagellates, ciliates and sarcodines are abundant

members of the protistan community (Buck & Garrison 1983, 1988, Marchant 1985, Garrison & Buck 1989a, b, Garrison et al 1991, Kivi & Kuosa 1994).

The nanoplanktonic haptophyte, Phaeocystis antarctica is arguable the single most abundant species of phytoplankton in Antarctic waters (Marchant 1993). P. antarctica forms dense, shallow blooms during austral spring, which can dominate the phytoplankton associated with the sea ice and in the MIZ (see above & Chapter 3). We shall also see (Chapter 4) that its blooms greatly influence the structure and function of the protistan community in Antarctic waters. Thus, P. antarctica ideally suits description as a key species of Antarctic marine phytoplankton. That blooms of this alga in Antarctic waters are often near-surface during spring indicate that it is likely to be exposed to increased levels of UVB radiation as a result of depletion of stratospheric ozone. Furthermore, the discovery that P. antarctica contained high concentrations of UV–absorbing compounds which afforded this species substantial protection from UVB radiation was instrumental in initiating the research contained within this thesis. Consequently, Chapter 3 provides a detailed examination of the biology and ecology of this remarkable alga.

CHAPTER 3

The biology and ecology of Phaeocystis (Haptophyceae)

1. INTRODUCTION

Phaeocystis Lagerheim is a marine phytoplanktonic genus in which four species are presently recognised: P. pouchetii (Hariot) Lagerheim; P. globosa Scherffel; P. antarctica Karsten; and P. scrobiculata Moestrup. Fryxell and Kendrick (1988), Sournia (1988) and Davidson and Marchant (1992b) indicated that the status of the species P. pouchetii was uncertain. Recent resurrection of P. globosa and P. antarctica (Medlin et al. 1994) bear-out this uncertainty. The study by Medlin et al. (1994) was, however, limited in its scope and the number of Phaeocystis species remains uncertain. In addition, while P. globosa and P. antarctica have now been resurrected, the prior taxonomic confusion has meant that authors have not differentiated between species. For the purposes of this thesis, P. scrobiculata is only referred to when specifically stated. All other species are collectively referred to as Phaeocystis unless the currently accepted taxonomic identity is known. Phaeocystis south of the Antarctic Convergence is referred to as P. antarctica.

The uniqueness of the genus Phaeocystis rests not only with the vast biomass attained by its blooms but with its exceptional physiology. Phaeocystis can dominate entire ecosystems with significant socio-economic impacts on fisheries, aquaculture and recreation. Phaeocystis has a world-wide distribution. In high latitudes their blooms can attain concentrations as high as 6×10^7 cells per litre (Davidson & Marchant 1992a), dominating the phytoplankton over distances greater than 100 miles (eg. Savage 1930, Hart 1942, Lancelot 1983, Barnard et al. 1984, Chang 1984, Fryxell & Kendrick 1988, Eilertsen 1989, Lutter et al. 1989, Davidson & Marchant 1992a). Because of its abundance and the diversity of its extracellular products, Phaeocystis is one of the most

important phytoplanktonic organisms in polar and subpolar seas (Lancelot et al. 1987, Davidson & Marchant 1992a). So conspicuous are blooms of Phaeocystis that it is one of the very few phytoplanktonic organisms for which colloquial names been coined; being known variously as "Tasman Bay slime" (Chang 1983), "baccy juice", "fisherman's sign", "weedy water" and "stinking water" (Orton 1923, Savage 1930).

Progressive eutrophication of the Wadden Sea has led to this alga increasing in abundance and lengthening the duration of its bloom to the extent that it has become a weed (Cadée & Hegeman 1974, 1979, 1986, Verity et al. 1988b). Blooms of Phaeocystis are avoided by fish, they clog commercial fishing nets (eg. Savage 1930, Chang 1983), are of low nutritional value and appear detrimental to the growth and reproduction of shellfish and metazooplankton (eg Walne 1970, Pieters et al. 1980). They also contaminate the sea floor and high tide mark with mucilage (Grontved 1960), which becomes anoxic with bacterial activity. This results in avoidance by fish and causes widespread mortality amongst the benthic infauna and littoral invertebrates (Rogers & Lockwood 1990). Sea foam, derived from the dissolved organic carbon released during collapse of Phaeocystis blooms, forms massive foam deposits that accumulate on beaches of Northern Europe (eg. Eberlein et al. 1985, Lancelot et al. 1987) and smother appendicularians and nematodes (Armonies 1989). Blooms also release prodigious quantities of dimethylsulfide which may contribute significantly to acid rain over Scandanavia (Pearce 1988). The economic ramifications of these blooms, coupled with their ecological significance, maintain this species as the focus of considerable research effort.

Phaeocystis remains an enigma with respect to its taxonomy, species succession, ecological role, antibiosis and the fate of its blooms (Fryxell & Kendrick 1988, Verity et al. 1988b). To this should be added its life cycle and aspects of its physiology. Thus, despite the obvious impacts of blooms of this alga (Lancelot et al. 1987, Davidson & Marchant 1992b, Rousseau et al. 1994) and the wealth of research published about it,

knowledge of its relationship with the biotic and abiotic environment remains relatively poor (Weisse et al. 1986).

There are two main stages in the life history, a biflagellate motile stage and a colonial stage. With few exceptions, blooms of Phaeocystis are composed of colonial stage cells. This stage confers intriguing abilities to which Phaeocystis must owe much of its prevalence. Transition from the flagellate to the colonial phase results in each cell losing part of its autonomy (Verity et al. 1988b). Each colonial cell exudes large quantities of its photoassimilated carbon (eg. Lancelot 1984a), most as mucopolysaccharides, which perform both a structural function by contributing to the colony matrix and a physiological role by acting as a carbon source in dark catabolism (Lancelot & Mathot 1985), a reservoir of phosphate (Veldhuis & Admiraal 1987) and a site of UV-B screening compounds (Marchant et al. 1991). Colony size and its low nutritional value result in decreased grazing pressure on this alga while its prolific release of compounds such as DMS (Andrae & Raemdonck 1983, Barnard et al. 1984, Gibson et al. 1990) and acrylic acid (Sieburth 1960) may also deter grazers (Estep et al. 1990) and hinder microbial decomposition (Sieburth 1960, 1961, Davidson & Marchant 1987).

2. TAXONOMY

Phaeocystis pouchetii and P. scrobiculata were the only species of Phaeocystis recognised until recently. However, taxonomic delimitation of P. pouchetii was uncertain (Parke et al. 1971, Fryxell & Kendrick 1988, Sournia 1988, Davidson & Marchant 1992b). Taxonomic problems were exacerbated by the life cycle being polymorphic and the life history incompletely known (Fryxell & Kendrick 1988, Sournia 1988, Rousseau et al. 1994). On the basis of differences in periplast scales and thread-like arrays or "stars" produced by its motile cells there is little doubt (see Sections 3.1, 3.2.1) that P. scrobiculata (Moestrup 1979) is a separate species. Recent taxonomic studies using molecular biological techniques have resurrected P. globosa Scherffel and P. antarctica

Karsten (Medlin et al. 1994). However, the full extent of speciation in this genus remains to be ascertained.

The first description of cells now known as Phaeocystis was made by Hariot in Pouchet (1892) as Tetraspora Poucheti sp. nov. Later, Lagerheim (1893) created the genus Phaeocystis then reclassified this alga as belonging to it (Lagerheim 1896). Nine species of Phaeocystis have been formally described. However, reviews of the genus (Kornmann 1955, Bourrelly 1957, Kashkin 1963) resulted in Phaeocystis globosa Scherffel, P. sphaeroides Buttner, P. amoeboidea Buttner, P. giraudyi (Derb. et Sol.), P. antarctica Karsten and P. brucei Mangin being relegated to synonymy with P. pouchetii because the nature and arrangement of cells within the colonies were not considered a criteria to justify their taxonomic separation.

The organism described as Procytella primordialis (Haeckel 1890) may well be P. pouchetii, but if synonymy were ever established, it is recommended that this name be ignored in favour of Phaeocystis as "nomen conservandum" (Sournia 1988). The species name P. poucheti occasionally appears in the literature (eg Kornmann 1955, Guillard & Hellebust 1971); however the original Poucheti was amended to pouchetii to comply with the orthographic conventions of the International Code of Botanical Nomenclature (Voss 1983, cited in Sournia 1988). Reference to Phaeocystis antarctica Karsten as a blue green alga (Richardson & Whitaker 1979) is erroneous. Its colonies, described as growing extensively in sea ice off Signy Island, are highly likely to be of Phaeocystis antarctica. The alga Corymbellus aureus Green is a colonial prymnesiophyte of similar cell size and gross morphology to Phaeocystis and it is possible that misidentification may have occurred (Fryxell & Kendrick 1988). The former, however, forms simple clusters of flagellate cells which are not bounded by a mucilaginous sheath (Green 1976).

Phaeocystis is a cosmopolitan genus with occurrences reported from the tropics (Estep et al. 1984, Margalef 1978, Al-Hassan et al. 1990) to both northern and southern polar waters (eg. El-Sayed et al. 1983, Barnard et al. 1984). It has also recently been isolated

by Hallegraeff and Blackburn (pers. comm.) from equatorial waters off Palau. Phaeocystis have thermally distinct strains (eg. Kornmann 1955, Kayser 1970, Grimm & Weisse 1985, Weisse et al. 1986, Marchant et al. 1991) and fill different ecological roles (eg. Colijn 1983, Palmissano et al. 1986, Fryxell & Kendrick 1988).

Phaeocystis globosa has long been a contender both for separate species status and potential taxonomic precedence over P. pouchetii. The description of P. globosa by Scherffel (1899, 1900) is more detailed than the description of P. pouchetii by Hariot in Pouchet (1892). Sournia (1988) considers that Scherffel better describes both a prymnesiophyte and Phaeocystis than do Hariot or Lagerheim's (1896) diagnoses. However, despite P. globosa being a more complete description of the species, P. pouchetii took taxonomic precedence and the former has fallen into disuse until recently. Studies by Rick and Aletsee (1989), Jahnke and Baumann (1986, 1987) and Baumann and Jahnke (1986) contend that differences in environmental optima and colonial morphology warrant the re-establishment of P. globosa as a separate species. The arguments presented for its re-establishment do not, however, contain ultrastructural data on the flagellate stage, upon which the taxonomy of the species of Phaeocystis are based. Numerous studies of the genus Phaeocystis have concluded that colony morphology was an insufficient criterion on which to separate Phaeocystis species and that the globosa - type colony was one of several morphological varieties of P. pouchetii (Kornmann 1955, Kashkin 1963, Parke & Dixon 1968, Chang 1983). Further, the suggestion by Rick and Aletsee (1989) that the geographic separation of the colony morphotypes in the North Sea with P. pouchetii to the north and P. globosa to the south was refuted by as early as 1930 by Savage who found pouchetii-type Phaeocystis in the Southern Bight of the North Sea. This led Ostenfeld to rescind his claim made in 1910 (cited Savage 1930) that the species distributions were mutually exclusive. Analysis of the elemental composition and growth dependence of pouchetii-type and globosa-type Phaeocystis by Jahnke (1989) did little more than demonstrate already documented differences in thermal strain and physiology that result from inhabiting different environments.

18S ribosomal RNA sequencing of Phaeocystis conducted by Medlin et al. (1994) substantiated separation of Phaeocystis pouchetii into three species; retaining P. pouchetii and resurrecting P. globosa Scherffel and P. antarctica Karsten. This study also indicated that the cold water forms (P. pouchetii and P. antarctica) evolved from the warm water species (P. globosa) approximately 50 million years ago and relates this to climatic and tectonic events such as the isolation of the Arctic basin from the rest of the world's oceans and commencement of the circum-Antarctic circulation. While separation of these species have now been established, the prior taxonomic confusion has meant that authors have not differentiated between them. Thus, with few exceptions, the true taxonomic identity of the Phaeocystis in northern hemisphere studies remains unknown. So too does the extent of biological and ecological differences between these species. The fact that they remained inseparable until now suggests a high degree of similarity. Thus, consideration of physiology or interaction of Phaeocystis with the biotic and abiotic environment in this review differentiates little between individual Phaeocystis species.

The chemotaxonomic study conducted by Medlin et al. (1994) was limited in scope as it contained only seven strains/species of Phaeocystis. Furthermore, the life history of the genus is poorly understood and its geographic range is enormous. Thus, more species of Phaeocystis may be recognised in the future. In Antarctic waters it remains unknown whether the morphologically similar P. globosa and P. antarctica coexist or whether other previously described species from Antarctic waters, such as P. brucei Mangin, are valid (Medlin et al. 1994). It is noteworthy that the related genus Chrysochromulina, which also has a global distribution, contains at least forty seven described species (Estep & MacIntyre 1989).

Baumann et al. (1994) augmented the separation of Phaeocystis species by Medlin et al. (1994) by defining the known structural, physiological and biochemical differences between these species. Differences cited include the morphology of the colonial stage, the thread-like arrays or "stars", organic scale shape and size and characteristics of the

haptonema and flagella of the single cells. Temperature tolerance and biochemical differences in the pigment and carbon content per cell are also cited. However, the lack of comprehensive data for all Phaeocystis species (Lancelot & Rousseau 1994) and the degree of overlap of characteristics between species renders them of limited value in routine identification where distributions overlap.

3. CELL STRUCTURE AND LIFE CYCLE

3.1. P. scrobiculata

It is yet to be reported whether P scrobiculata has a colonial stage and if so whether it is distinguishable from other Phaeocystis species. The only reports of this species are as preserved flagellates. These differ from other Phaeocystis species in the structure and arrangement of its periplast scales and the arrangement of its thread-like stars (Fig. 1A, Table 1) (Moestrup 1979, Hallegraeff 1983).

3.2. Phaeocystis spp.

No description of the ultrastructural of P. antarctica has been published and the only information available is that reported by Davidson (1985). The life cycle has only been reported for P. globosa (Rousseau et al. 1994) and this is only partly resolved. It is known to contain at least two different planktonic phases and may also include a benthic stage (Kornmann 1955, Kayser 1970, Verity et al. 1988b, Rousseau et al. 1994). Each of these phases is apparently capable of vegetative reproduction. However, differentiation of one phase from another is not fully understood and surprisingly little ultrastructural work has been reported.

Table 1. Scale and star morphology of Phaeocystis species. * refers to data from Moestrup 1979.

Phaeocystis Species	"Stars"	Scale Type	Scale Dimensions (μm)	Scale Position (relative to cell)	Scale Patterning
P. scrobiculata (Moestrup 1979, Hallegraeff 1983)	9 rayed	Large oval	0.6 x 0.45* or 0.41 x 0.3	Distal	Ventral: ridges radiating from plain centre. Dorsal: no decoration
		Small circular-oval	0.21 or 0.1	Proximal	Ventral: ridges radiating from plain centre. Dorsal: no decoration, patternless rim
P. globosa (Parke et al. 1971)	5 rayed - proximal ends membrane bound	Large circular-oval	0.18 x 0.19	Distal	Outward raised rim. ~48 ridges radiating from rectangular plain centre on both surfaces.
		Small oval	0.10 x 0.13	Proximal	Inflexed rim. ~30 ridges radiating from rectangular plain centre on both surfaces.
P. pouchetii	?	?	?	?	?
P. antarctica (Davidson 1985)	5 rayed - proximal ends membrane bound	Large circular-oval	0.19 x 0.18	Distal	Outward raised rim. ~45 ridges radiating from rectangular plain centre on both surfaces.
		Small oval	0.08 x 0.12	Proximal	Inflexed rim. Ridges radiating from rectangular plain centre on both surfaces.

3.2.1. Flagellate cells

Using isolates from the English Channel, Parke et al. (1971) provide the only detailed ultrastructural description of Phaeocystis motile cells and this was probably of P. globosa (Baumann et al. 1994). These have an anterior depression, two golden brown plastids, the thylakoids of which are arranged in stacks of three with a girdle lamella. The two flagella are of equal length bearing hair points. Arising between the flagella but in a different plane from the flagella bases is a short, stiff, bulbous ended haptonema (Fig. 2A, B). Cells bear a periplast of two layers of organic scales and threads arranged as a five armed "star" array. These differ in structure and arrangement from those of P. scrobiculata. However, the few ultrastructural studies suggest they differ little between other species of Phaeocystis (Table 1) (Parke et al. 1971).

Each constituent thread of the "stars" is up to 20 μm long, tapering towards its tip (Parke et al. 1971). The proximal ends form a pentagon bounded by a membrane (Fig. 1B). The synthesis, release and function of the stars remains unsure. I have found in shadow cast preparations of Antarctic material that the threads are tubular in section and appear to consist of overlapping segments as well as single pieces (Fig. 1C). The flagellate cells reportedly possess at least two anterior, membrane bound discs which are circular to oval in shape and contain the threads (Parke et al. 1971, Pienaar 1991). Parke et al. (1971) described Phaeocystis as possessing discs which may either become, or are released into, prominent vesicles which protrude from flagellate cells. Similarly, Davidson (1985) reports the occurrence of stars within these prominent vesicles, finding that threads were regularly arranged closely oppressed to the inside of the vesicle membrane of P. antarctica. Once the vesicle of Phaeocystis ruptures the arrays are released from the cells giving the characteristic appearance of threads apparently coiled about the cell (Parke et al. 1971, Hallegraeff 1983, Davidson 1985) (Fig. 2B). The arrays presumably then separate and the arms straighten, adopting their characteristic configuration (Fig. 1B, 2A).

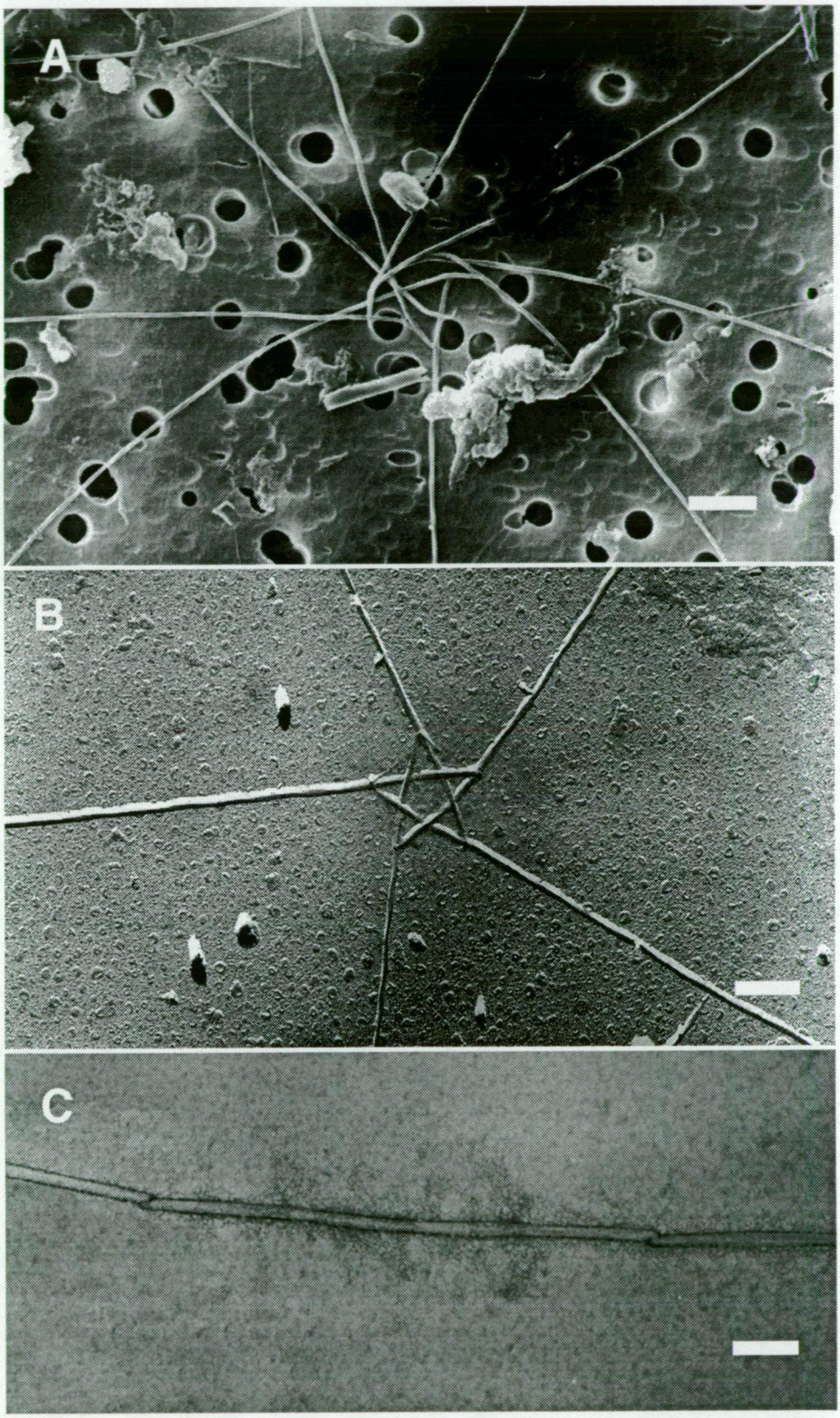


Fig. 1. Electron micrograph of (A) Phaeocystis scrobiculata star (micrograph from Dr. G. Hallegraeff); (B) Phaeocystis antarctica star (micrograph from Dr. H. Marchant), and (C) detail of star arm showing the presence of overlapping segments. Scale bars = 2μm, 500 nm and 300 nm respectively.

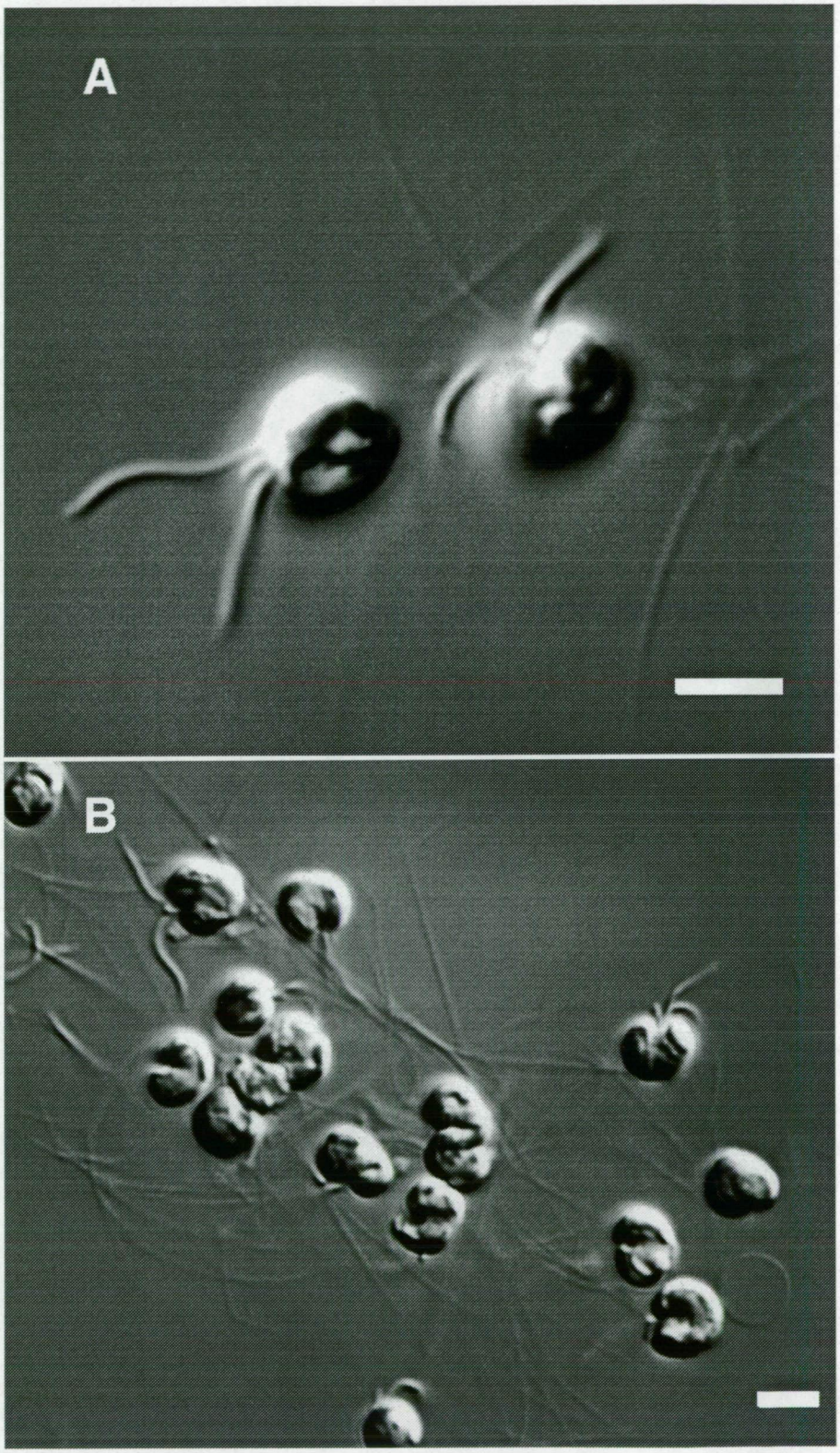


Fig. 2. Nomarski light micrograph of the flagellate stage of P. antarctica showing (A) flagella, haptonema and five armed stars, and (B) cells surrounded by thread-like star arms (scale bars = 5μm)

Cellular structures of Phaeocystis including the pentagonal stars, scales and flagella exhibit very little variation over the organism's range (Booth et al. 1982, Buck & Garrison 1983, Hallegraeff 1983, Davidson 1985, Marchant & Nash 1986, Lancelot et al. 1987, Perrin et al. 1987). Reports indicate variation in the length of star arms which may reach 50 μm (Buck & Garrison 1983, Lancelot et al. 1987) to 100 μm (Fryxell 1989) and circular plate scales which reach 0.25 μm diameter (Hallegraeff 1983).

Kornmann (1955) suggested that the flagellates may be subdivided into three types, all of which are capable of vegetative multiplication namely:

1. An asexual "swarmer" (between 4.5 to 8 μm diameter).
2. A microzoospore (3 to 5 μm).
3. A macrozoospore.

Doubt exists as to the validity of these as separate stages. The macrozoospore is of unspecified size but reportedly produce colonial cells (Rousseau et al 1994). Kornmann (1955) provides the only other record of this stage but claims that Scherffel (1899) also described a colony containing macrozoospores. Essentially all recent investigations fail to distinguish between the remaining two flagellate stages of Phaeocystis on the basis of size. Flagellates reportedly range in size from 3 to 8 μm (Kornmann 1955, Parke et al. 1971, Buck & Garrison 1983, Hallegraeff 1983, Fryxell 1989, Lancelot et al. 1987), the same as that reported by Verity et al. (1988b) for colonial cells. However, Kornmann (1955) proposed that this range in cell size contains the microzoospore and swarmer cell stages.

The existence and importance of the microzoospore in the life cycle of Phaeocystis has been demonstrated in flow cytometric studies by Cariou et al. (1994). These studies were conducted on P. globosa and showed that microzoospores, which were formed during the senescent decline of colonies, were haploid. In contrast, motile swarmers and non-motile cells derived from swarmers were diploid (Cariou et al. 1994, Rousseau et al. 1994). This is the first indication of sexuality in the life cycle Phaeocystis.

Microzoospores have also been observed for P. antarctica (Davidson unpubl.). It is likely

that these are also haploid but their ploidy has not been ascertained. The process of microzoospore formation and the sexual process that forms the diploid non-motile or swarmer cell stages are not known. It is also unknown whether there is alternation between haploid and diploid colonies. Sexuality as a potentially common occurrence in the life cycle of Phaeocystis spp. would lend genetic plasticity to members of the genus and could help explain its world-wide distribution (Rousseau et al. 1994).

3.2.2. Non-motile single cells

In addition to these flagellate cells, Kornmann (1955), Kayser (1970) and Rousseau et al. (1994) report a solitary non-motile cell type. Kornmann (1955) described the formation of this stage from flagellate cells while Rousseau et al. (1994) report their formation by colonial the colonial stage. Kornmann (1955) reported that the non-motile cells formed colonies. In contrast, Kayser (1970) reported that this stage attaches to solid surfaces and releases new free single cells and colonies into the water column. These non-motile single cells were proposed as being benthic or attached to motile particles in nature. Whether or not all authors refer to the same cell type or Phaeocystis species is uncertain.

3.2.3. Colonial Cells

It is in the colonial form ("palmella"-stage) that Phaeocystis blooms and is most conspicuous. Colonies may reach 2 cm in diameter (Gieskes & Kraay 1975, Verity et al. 1988a) and exhibit the physiological peculiarities for which the species is renowned. In spite of the widespread occurrence and ecological importance of the colonial stage, there is only one report of its ultrastructure (Chang 1984). Colonial cells lack body scales, haptonema and flagella and are embedded in a mucilaginous matrix (Fig. 3A, B, C & D). Chang (1984) proposes that chrysolaminarin vesicles, which he observed protruding through the plasmalemma, deposit a multi-layered mucilaginous envelope. Our observations of colonial Phaeocystis antarctica have revealed no indication of multiple

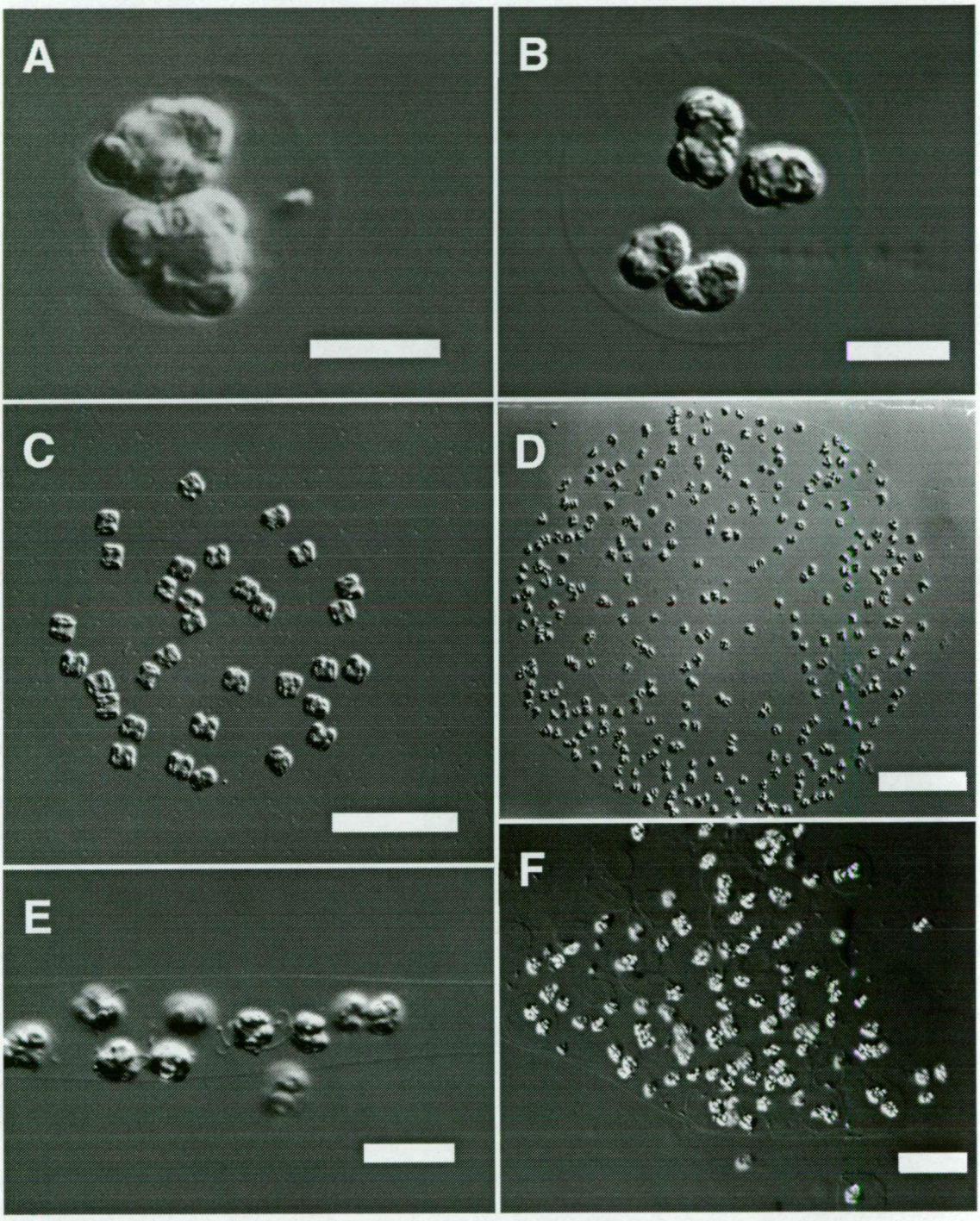


Fig. 3. Nomarski light micrograph of P. antarctica colonies showing (A), (B), (C) and (D) a range of colony diameter sizes (scale bars = 5, 5, 20 and 50 µm respectively), (E) a section of an elongate colony in which cells are differenciating into the flagellate stage (scale bar = 15µm) and (F) part of a large senescent colony in which small colonies are being formed (scale bar = 30µm).

mucilaginous layers. Chang (1984) claims that in all other respects this stage is indistinguishable from the flagellate stage.

The ultrastructural variability of colonial Phaeocystis over its range remains unknown but gross colony structure and arrangement of cells can be extremely variable. Colonies range in size from approximately 10 μm containing a single cell to 2 cm containing thousands of cells (Gieskes & Kraay 1975, Verity et al. 1988a) (Fig. 3A, B, C & D). P. globosa and P. antarctica colonies are characteristically spherical with cells randomly distributed within (Fig. 1A, B, C, D). P. pouchetii colonies may be elongate, ovoid, (Fig 3E) or lobed (Kornmann 1955, Bätje & Michaelis 1986) with cells clumped, usually in groups of four cells. Colonies can, however, vary greatly and may occur as irregular flat discs (Kayser 1970) or dense aggregated mats (Chang 1983, Nichols et al. 1991). Cells may be aggregated at one pole (Davidson unpubl.) or bereft of cells as a result of life-stage changes and emigration of cells as flagellates (Verity et al. 1988b). All of these colony morphologies and cell arrangements have been observed for P. antarctica in cultures established from a single colony (Davidson unpubl.), suggesting a high degree of plasticity in colony morphology and/or strain/species variation between isolates.

The cells of the colonial stage secrete 5-80% of their photoassimilated carbon (eg. Guillard & Hellebust 1971, Gieskes & van Bennekom 1973, Colijn 1983, Lancelot 1983, Laandbroek et al. 1985, Veldhuis et al. 1986a), much of which is devoted to matrix formation (Lancelot & Mathot 1987). The rewards for this metabolic expense of colony formation are apparently considerable. The colony creates its own microenvironment in which it can manipulate trace metal concentrations (Lubbers et al. 1990), protect against bacterial attack (Davidson & Marchant 1987), store photosynthate for catabolism in the dark (Lancelot & Mathot 1985) and retain UV-B absorbing compounds which shield the cells from damaging wavelengths (Marchant et al. 1991). Furthermore, the colony tends to function as a biological entity rather than a passive aggregation of cells (Lancelot & Mathot 1985, Veldhuis & Admiraal 1985, Lancelot et al.

1986, Veldhuis et al. 1986a). Verity et al. (1988a) showed that the colonies had endogenous regulation of formation, growth, senescence (Fig. 3F) but more importantly were capable of cleavage to produce smaller daughter colonies. Such sophisticated organisation of the colony is reminiscent of the green algal order Volvocales (Verity et al. 1988a).

3.2.4. Life Cycle Changes

Differentiation of cell stage by Phaeocystis in culture is not entirely predictable. The currently known stages and relationships between stages in the life cycle of Phaeocystis are presented in Figure 4. Flagellate cells may persist indefinitely by vegetative reproduction, can be released by colonies (Fig. 3E) and the "swarmers" may reform colonies (Kornmann 1955, Kayser 1970, Parke et al. 1971, Verity et al. 1988b, Fryxell 1989) (Fig. 3F). However, the regulation of differentiation is poorly understood. Colonial cells that experience a rapid decrease in temperature or chronic nutrient deprivation change to the flagellate cell stage and exit the colony (Verity et al. 1988b, Davies et al. 1992). Once initiated this process is irreversible. This exodus probably accounts for the high numbers of flagellates observed following the decline of colonial blooms (Veldhuis et al. 1986b, Davidson & Marchant 1992a) and may help explain the apparent sudden disappearance of colonial Phaeocystis blooms (Orton 1923, Admiraal & Venekamp 1986). Similar to colonial cultures (see Section 3.1), colonial blooms in the field reportedly release "swarmers" (Jones & Haq 1963) or microzoospores (Veldhuis et al. 1986b, Verity et al. 1988b). Non-motile unicells may also contribute to the formation of new colonies (Chang 1983).

The relative abundance of flagellates and colonies can be controlled by nutrient availability. Eutrophication (Guillard & Hellebust 1971) or high phosphate concentration alone (Veldhuis & Admiraal 1987) can induce the dominance of flagellates in culture. In comparison with the flagellates the colonial stage is reportedly a poor competitor under phosphate or ammonia limitation (Reigman et al. 1992, Cariou et al. 1994, Rousseau et

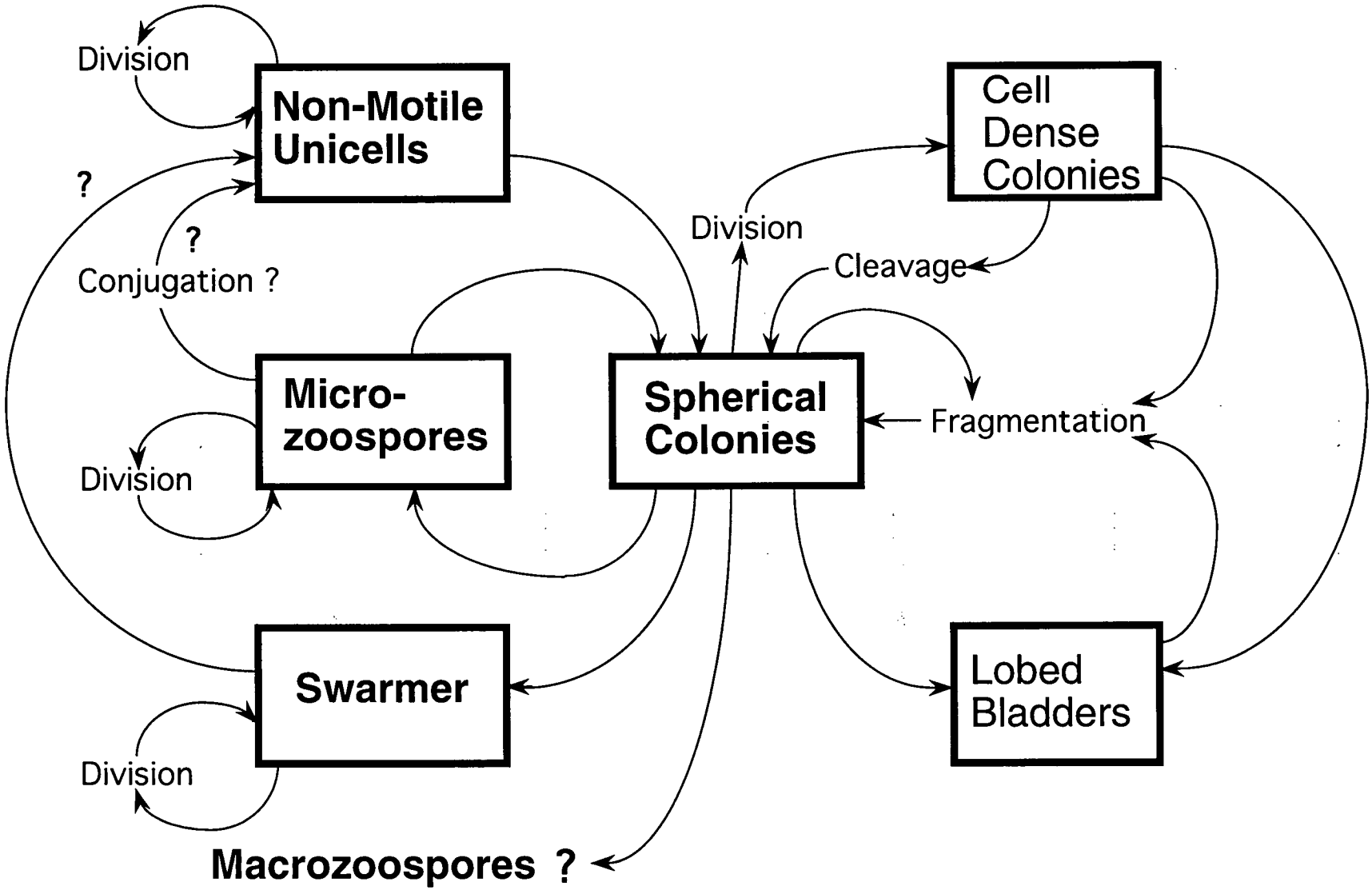


Fig. 4. Current known stages and relationships between stages in the life cycle of Phaeocystis (after Veldhuis et al. 1987).

al. 1994) but dominated in nitrogen controlled environments (Reigman et al. 1992, Rousseau et al. 1994). Both high (>5 μM) and low (<0.3μM) phosphate concentrations appear inhibitory to development of the colonial stage, thus flagellates dominate. Between these extremes exists a nutrient concentration window in which colonies flourish (Cariou et al. 1994). Anthropogenic enrichment of Dutch coastal waters, especially with phosphate, has changed the limiting macronutrient from phosphate to nitrogen. This change may explain the development of massive "nuisance" blooms of colonial Phaeocystis sp. in the region (Reigman et al. 1992). Differentiation of colonial cells to flagellates by high phosphate concentration can be inhibited by simultaneous addition of soil extract (Veldhuis & Admiraal 1987). However, the identity of the constituent/s of soil extract that influence differentiation are unknown. The change from the flagellate to colonial stage may also be stimulated by exudates from diatoms (Boalch 1987).

Paperzak (1993) proposed that alternation between the colonial and flagellate stages in the life cycle of Phaeocystis were controlled by irradiance. Irradiances above 100 Watts h m^{-2} day^{-1} supported the colonial life stage and below this irradiance supported the flagellate. This control was used to explain bloom initiation and the release of flagellates at the end of the bloom. Loss of buoyancy control by colonies during senescence would result in sinking and the consequent decline in irradiance would initiate release of the flagellate stage (Paperzak 1993).

The over-wintering strategy of Phaeocystis is unclear. Davies et al. (1992) suggested that in the Southern North Sea it over-winters on the sea bed. Penetration of enough light to allow proliferation of these benthic cells the following spring (Joint & Pomeroy 1993) and grazing of Phaeocystis by benthic organisms suggests such an over-wintering strategy is unlikely. Only its flagellate stage is commonly observed to persist in the phytoplankton throughout the year (Parke et al. 1971, Boalch 1987). Their hardiness led Kornmann (1955), Nøst-Hegseth (1982), Boalch (1987), Veldhuis et al. (1986b) and Verity et al. (1988b) to proposed that the flagellate represents a spore-like stage, a refuge

from conditions stressful or lethal to the colonial stage. In contrast, this stage is capable of rapid vegetative reproduction (Kornmann 1955, Kayser 1970, Verity et al. 1988b), occasionally comprising the majority of the Phaeocystis bloom (Morris 1971), and by returning to the colonial stage, may contribute to the colonial blooms (Kornmann 1955, Jones & Haq 1963, Cadée & Hegemann 1986, Tande & Båmstedt 1987, Veldhuis & Admiraal 1987, Eilertsen 1989, Fryxell 1989, Davidson & Marchant 1992a). Such behaviour better befits description as escape from the confines of colonial metabolism to a proliferation and dispersal phase rather than a "spore" or "refuge". Alternatively, Jones and Haq (1963), Kayser (1970) and Verity et al. (1988b) suggest that the flagellate may be part of more complex life cycle changes that result in the development of resistant spores. The attached solitary cell stage has also been proposed as over-wintering form of Phaeocystis (Kayser 1970, Sieburth 1979), a life cycle strategy that would, according to Sieburth (1979), restrict this alga to inshore or coastal waters.

4. DISTRIBUTION AND ABUNDANCE

Phaeocystis scrobiculata has been reported off New Zealand where it was first described (Moestrup 1979), in the East Australian Current (Hallegraeff 1983) and from the equatorial Atlantic (Estep et al. 1984) (Fig. 5). The infrequency of reported occurrences of this species may reflect a limited geographic range, low abundance, or problems of distinguishing it from other Phaeocystis species.

Other species of Phaeocystis are a minor component of the phytoplankton in warm temperate and tropical waters (Fig. 5). Guillard and Hellebust (1971) found that a tropical isolate of this alga from waters off Surinam grew rapidly in culture and noted, in the light of this finding, that the relative absence of Phaeocystis in warm neritic waters was unexpected. Al-Hassan et al. (1990) subsequently reported a bloom of Phaeocystis in Kuwait Bay in the Arabian Gulf, demonstrating that blooms of the species are not excluded from tropical waters. Interestingly, Al-Hassan et al. (1990) concluded that such near-surface summer blooms of Phaeocystis were probably due to nutrient enrichment by

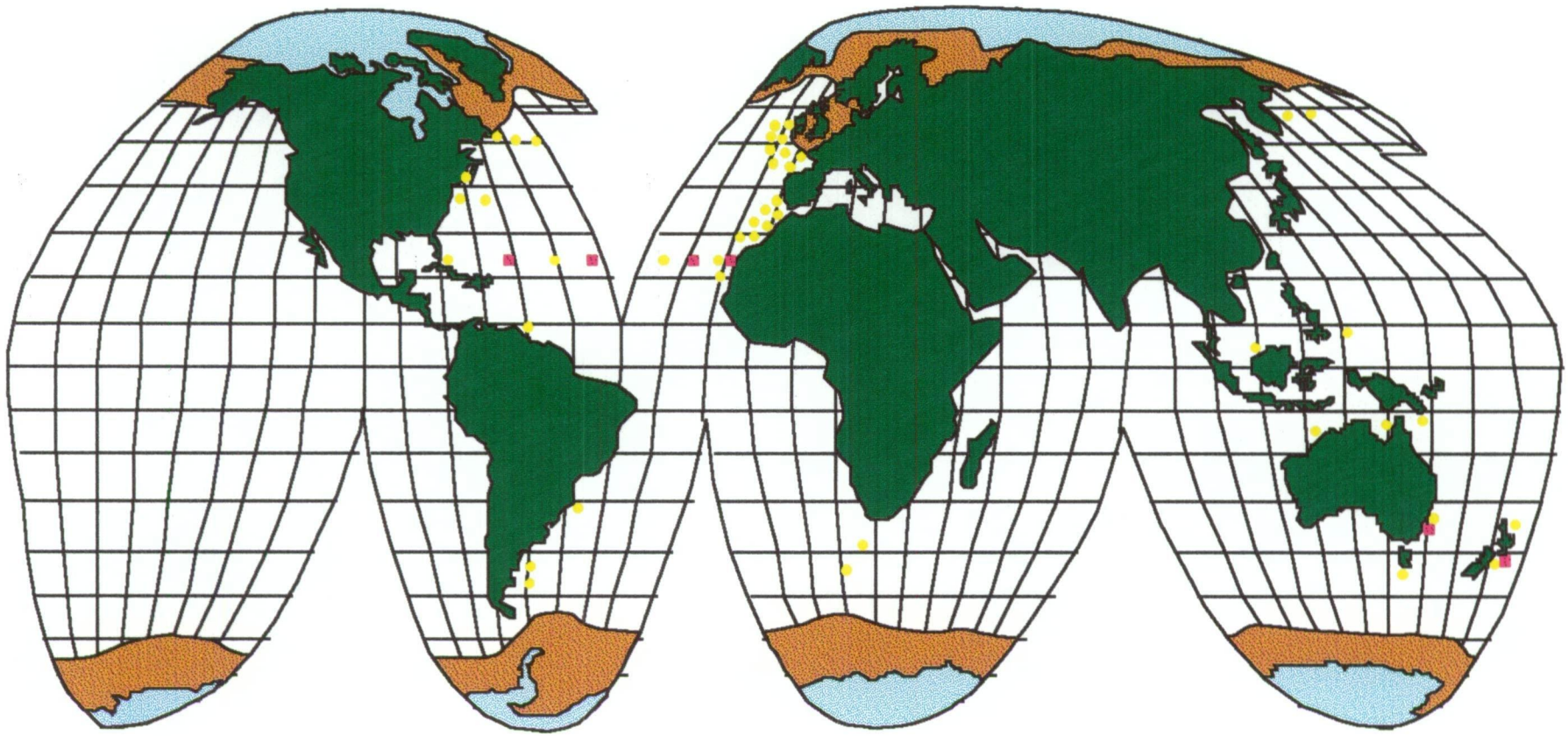


Fig. 5. The global distribution of Phaeocystis showing its widespread occurrence in polar and cool temperate waters (gold regions). Single records are denoted by yellow circles, and occurrences of Phaeocystis scrobiculata by red squares. Records from tropical and temperate waters largely reflect the distribution of observers rather than that of Phaeocystis.

industries and sewage. Thus, in the absence of nutrient enrichment by human activity (Al-Hassan et al. 1990) or upwelling (Margalef 1978), oligotrophic tropical waters may not be an environment conducive to the development of its blooms. In comparison with the flagellate stage Verity et al. (1991) found that changes in photosynthesis and cellular excretion rates as a result of high light and temperature stressed the colonial stage of Phaeocystis. This they suggested may explain the relative absence of Phaeocystis colonial blooms in tropical and subtropical waters. Observations of Phaeocystis by Al-Hassan et al. (1990), Estep et al. (1984), Atkinson et al. (1978), Margalef (1978), Hallegraeff (1983), and Hallegraeff and Blackburn (pers. comm.) have extended the range of this species in the tropics beyond that reported in Kashkin (1963).

Phaeocystis is most abundant in polar waters of both the northern and southern hemispheres (eg. Savage 1930, Lucas 1940, Hart 1942, Jones & Haq 1963, Kashkin 1963, Iverson et al. 1979, Eilertsen et al. 1981, Bölter & Dawson 1982, Booth et al. 1982, Chang 1983, von Bodungen et al. 1986, Garrison et al. 1987, Fryxell & Kendrick 1988, Verity et al. 1988a, Holm-Hansen et al. 1989a) (Fig. 5). Its blooms frequently dominate the phytoplankton (eg. Joint & Pomeroy 1981, Buck & Garrison 1983) where it is reported by various authors as contributing 40-83% of the total phytoplankton numerical abundance during the productive period (Eilertsen et al. 1981, Nøst-Hegseth 1982), in excess of 90% of the total phytoplankton cell number at its peak (Joiris et al. 1982, Lancelot 1984b, Lancelot et al. 1986, Veldhuis et al. 1986b), and 65% of the annual primary production (Joiris et al. 1982). In polar waters it is often the first species to bloom (eg. Hart 1942, Lutter et al. 1989, Davidson & Marchant 1992a) and frequently dominates the algal community within the sea ice (eg. Fryxell et al. 1984, Garrison et al. 1987, Garrison & Buck 1989b, Gradinger & Baumann 1991, Kang & Fryxell 1993) from where it may seed the water column (Fryxell et al. 1984, Fryxell & Kendrick 1988, Garrison & Buck 1989b, Gradinger & Baumann 1991, Kang & Fryxell 1993) to dominate the ice-edge blooms (eg. Buck & Garrison 1983, Garrison et al. 1987, SooHoo et al. 1987, Gradinger & Baumann 1991, Kang & Fryxell 1993). The flagellate stage of

P. antarctica is also a major contributor to the ice edge and open water phytoplankton community during the austral winter (Ashworth et al. 1990).

In temperate waters the bloom of Phaeocystis usually occurs after that of diatoms (eg. Jones & Haq 1963, Gieskes & Kraay 1975, Cadée & Hegeman 1979, Colijn 1983, Cadée 1982, Weisse et al. 1986, Veldhuis et al. 1986b, Lancelot et al. 1987). Here it has received much attention from waters of the North Sea where its conspicuous blooms have been known since last century (Pouchet 1892, Scherffel 1899, 1900, Gran 1902, Wulf 1934, Lucas 1940, Künne 1952, Kornmann 1955, Eberlein et al. 1985, Owens et al. 1989, Riegman et al. 1990). These blooms may turn the sea oily, brown and smelly over areas more than 170 km across (Savage 1932) but are usually shortlived, collapsing a few days after their peak (Rogers & Lockwood 1990). Legendre (1990) proposed that avoidance of Phaeocystis by grazers (see Section 5) contributes to the widespread high concentrations of this species.

Continuous plankton records from the North Sea indicate that the abundance of Phaeocystis has changed significantly between 1946 and 1987 (Reid 1975, Owens et al. 1989). In the northeast Atlantic Ocean and North Sea its abundance of has declined considerably from their maximum in the late 1940's and autumnal and winter occurrences, which were frequent before the mid 1950's, have largely vanished. Van Bennekom et al. (1975) and Gieskes and Kraay (1975, 1977) also report a decline in Phaeocystis abundance between the 1950's and 1970's. Since 1980 though, the occurrence of Phaeocystis has apparently increased again. Owens et al. (1989) observed a three yearly cycle in Phaeocystis abundance that was due to large scale physical changes which impacted the entire phytoplankton and zooplankton communities. In contrast, Phaeocystis abundance in northern European coastal waters, particularly in the region of the Wadden Sea, appears to have increased (eg. van Bennekom et al. 1975, Cadée 1986, Cadée & Hegeman 1986, Lancelot et al. 1987). It appears that anthropogenic nutrient enrichment of stratified coastal waters off northern Europe (eg. Foster et al. 1983,

Lancelot et al. 1987) is causing Phaeocystis blooms to attain higher numbers and persist longer (Cadée & Hegeman 1986, Lancelot et al. 1987, Cadée 1991). In high latitudes, similar to occurrences in enriched waters, Phaeocystis attains very high numbers and numerically dominates the phytoplankton for most of the growing season (Eilertsen et al. 1981, Davidson & Marchant 1992a).

Phaeocystis is most abundant in spring but also occasionally appears in lesser numbers in autumn (Savage 1930, 1932, Jones & Haq 1963, Cadée & Hegeman 1986, Owens et al. 1989). In contrast, Eilertsen et al. (1981) reported a remarkable year-round occurrence of Phaeocystis in Balsfjord, spanning a temperature range of 1 - 7°C while Verity et al. (1988a) also reported a winter bloom off Rhode Island. Savage (1930) mentions the occurrence of winter blooms in the North Sea. However, Phaeocystis colonies have reappeared as a feature of the winter phytoplankton in the Wadden Sea (Cadée & Hegeman 1986). This may again reflect the eutrophication of these waters. The seasonal span encompassed by this species again underlines its environmental plasticity.

Blooms of Phaeocystis are frequently reported to occur close to the surface (eg. Margalef 1978, Eilertsen et al. 1981, Palmissano & Sullivan 1985, von Bodungen et al. 1986, Fryxell & Kendrick 1988, Colijn et al. 1990) and many of its physiological adaptations point to it being well suited to a high light climate (see Section 6.4). In marked contrast, El-Sayed et al. (1983) found P. antarctica from the surface to 150 m depth adjacent to the Ross Ice Shelf, Antarctica. Here 25% of the total primary production in the water column occurred below the depths of 1% light penetration. In addition, Palmissano et al. (1986) found this alga could adapt to as little as 16 $\mu Em^{-2}s^{-1}$ light when advected beneath sea ice. Thus, although characteristically recorded as a species that forms near-surface blooms, P. antarctica is not restricted to these depths.

5. GRAZING

In northern European waters Phaeocystis blooms may contribute up to 99% of the total phytoplankton cell number (Lancelot 1984b) and 65% of the annual primary production (Joiris et al. 1982). Reports from polar and subpolar waters indicate it plays a similarly important role (eg. Bölter & Dawson 1982, El-Sayed et al. 1983, von Bodungen et al. 1986, Palmissano et al. 1986, Fryxell & Kendrick 1988, Davidson & Marchant 1992a). Such a major source of carbon is of considerable significance to higher trophic levels but evidence of the trophic fate of Phaeocystis appears contradictory. The complex sequence of morphotypes in the life cycle of Phaeocystis may facilitate escape from grazing by great changes in particle size (Putt et al. 1994). Furthermore, in excess of 80 - 90% of Phaeocystis colonies may be comprised of mucilage (Rousseau et al. 1990) and this material is apparently depauperate in nitrogen, phosphorus and nutrition (Billen & Fontigny 1987, Lancelot et al. 1987). Conversely, it may also facilitates selective feeding on specific types of Phaeocystis by a variety of heterotrophic organisms (Lancelot & Rousseau 1994).

Phaeocystis is variously reported to be of low nutritional value (Sargent et al. 1985, Al-Hassan et al. 1990, Claustre et al. 1990, Nichols et al. 1991, Bautista et al. 1992, Virtue et al. 1993b), incapable of supporting copepod growth and reproduction (Walne 1970, Gabbot & Walker 1971, Verity & Smayda 1989), avoided by invertebrates and fish (Orton 1923, Savage 1930, 1932, Bradstock & MacKenzie 1981, Martens 1981, Chang 1983, Schnack 1983, Ainley et al. 1986, Verity & Smayda 1989, Hansen et al. 1990, Rogers & Lockwood 1990), inefficiently grazed (Dagg et al. 1982, Daro 1985, Verity & Smayda 1989, Hansen et al. 1990, Hansen & van Boekel 1991, Davies et al. 1992, Bautista et al. 1994, Marchant & Thomsen 1994) or incapable of being grazed (Pieters et al. 1980). In contrast, it is also reported that Phaeocystis is grazed by protozoa (Admiraal & Venekamp 1986, Weisse & Scheffel-Möser 1990, Davidson & Marchant 1992a, Hansen & van Boekel 1991, Hansen et al. 1993) and metazooplankton (Lebour 1922,

Nicholls 1935, Sieburth 1960, Marr 1962, Jones & Haq 1963, Fretter & Montgomery 1968, Schnack 1983, Weisse 1983, Marchant & Nash 1986, Huntley et al. 1987, Tande & Båmstedt 1987, Sargent & Falk-Petersen 1989, Weisse & Scheffel-Möser 1990, Hansen et al. 1994), that its blooms are not avoided (Jones & Haq 1963, Fryxell et al. 1984, Weisse et al. 1986) and may provide a substantial proportion of the carbon necessary to support heterotrophic production (Lutter et al. 1989).

5.1. Shellfish

Feeding studies have shown that few grazers are incapable of ingesting Phaeocystis. Pieters et al. (1980) reported that the feeding apparatus of Mytilus edulis became clogged with colony mucilage and that this probably resulted in starvation. Phaeocystis was also found to be a poor food for adult oysters (Gabbott & Walker 1971) and resulted in reduced bivalve spawning success (Walne 1974, Pieters et al. 1980). This suggests that the ciliary feeding mode of the bivalves is poorly suited to the ingestion of mucilaginous material. Blooms of this alga did not, however, reportedly cause adult bivalve mortality in British or New Zealand waters (Gabbott & Walker 1971, Pieters et al. 1980, Bradstock & Mackenzie 1981) indicating that normal feeding could resume after the Phaeocystis bloom receded.

5.2. Fish

Phaeocystis blooms, though reportedly not directly toxic to fish (Parke et al. 1971), are also avoided by fish (Orton 1923, Savage 1930, 1932, Bradstock & MacKenzie 1981, Chang 1983, Rogers & Lockwood 1990). Savage (1932) refers to the slimy and probably unpalatable character of its blooms. Rogers and Lockwood (1990) proposed that the sea floor becomes covered with an anoxic layer of senescent Phaeocystis. This was avoided by juvenile flatfish and caused widespread mortality amongst benthic infauna and littoral invertebrates. No other authors have ventured reasons for the observed avoidance.

5.3 Metazooplankton

While euphausiids, copepods, cladocerans and meroplanktonic larvae have been shown to graze Phaeocystis (Lebour 1922, Nicholls 1935, Jones & Haq 1963, Fretter & Montgomery 1968, Hansen et al. 1994), the extent of their grazing provides a less coherent picture than those so far discussed. Blooms of this species are often massive but shortlived. The reproductive response time of metazoa, particularly at low temperatures, renders it highly improbable that such grazers as copepods could optimally utilise such a temporally transient substrate (Verity & Smayda 1989). Further, Weisse et al. (1986) states that the coincidence of Phaeocystis blooms with an absence of copepods observed by Martens (1980, 1981) was not a causal one but instead reflects development of their populations in time. This may also explain the declining copepod numbers observed by Smayda (1973) during a Phaeocystis bloom.

A recurrent result of Phaeocystis grazing studies is the determination that it is a suboptimal substrate. Biochemical analyses have shown that, in comparison with other phytoplankton, especially diatoms, Phaeocystis contain low concentrations of polyunsaturated fatty acids, neutral lipids, essential fatty acids and vitamin C (Sargent et al. 1985, Claustre et al. 1990, Priscu et al. 1990, Nichols et al. 1991, Virtue 1993b). This may partly explain reports of poor growth and reproduction in shellfish (Walne 1970, Gabbott & Walker 1971, Pieters et al. 1980) and copepods (van Rijswijk et al. 1989, Verity & Smayda 1989). However, Virtue et al. (1993a) suggests that Euphausia superba may be able to compensate for the low nutritional quality of Phaeocystis antarctica by using chain-elongation and desaturation of fatty acid to convert fatty acid components lacking in Phaeocystis to those required for metabolism. It is unknown whether other grazers are capable of fatty acid conversion.

The release of organic substances by Phaeocystis, particularly DMS (Barnard et al. 1984, Gibson et al. 1990), acrylic acid (Seiburth 1960, Guillard & Hellebust 1971) and large quantities of carbohydrate (Guillard & Hellebust 1971, Lancelot 1983), may deter

organisms from grazing it (Verity & Smayda 1989, Rogers & Lockwood 1990, Hansen & van Boekel 1991, Bautista et al. 1994, Davies et al. 1992, Marchant & Thomsen 1994) or occupying the same watermass (Smayda 1973, Martens 1980, 1981). Verity and Smayda (1989), refute this proposition on the basis that if colonies are ingested by some large suspension feeding copepods they cannot be chemically undesirable to these species and may not be to any species. Recently however, Estep et al. (1990) found in field studies that predation on Phaeocystis colonies by three copepods species was dependant upon the physiological state of the alga. The copepods Calanus finmarchicus, C. glacialis and C. hyperboreus avoided actively photosynthetic colonies but ingested senescent ones. Estep et al. (1990) also attributed this avoidance to the release of chemical deterrents by actively growing Phaeocystis, possibly acrylic acid or DMS. These results are at variance with those of Tande and Båmstedt (1987) and Hansen et al. (1990) who found the same copepod species actively grazing freshly cultured Phaeocystis.

As discussed earlier (see Section 3.2.1), flagellate Phaeocystis are 3 to 8 μm in diameter (Kornmann 1955, Kayser 1970, Parke et al. 1971). The nutritional value of the flagellates is equivalent to Chaetoceros for Calanus spp. (Tande & Båmstedt 1987). Nichols et al. (1991) showed that the flagellate had a higher lipid and fatty acid content than the colonial stage, making it a better source of nutrition. The size of colonies ranges from little larger than the flagellate to 2 cm in diameter. Thus, alternation by Phaeocystis between the colonial and flagellate stages allows changes in size of over three orders of magnitude (Parke et al. 1971, Gieskes & Kraay 1975, Verity et al. 1988a). This would have profound effects on the capacity of zooplankton to graze its blooms and the efficiency with which they are grazed, potentially making this alga relatively unavailable to metazooplankton (Reynolds et al. 1982, Verity et al. 1988b).

Schnack (1983) and Miller and Hampton (1989) reported that omnivorous copepods and those with a "mixed and raptorial feeding mode" in the Southern Ocean grazed P. antarctica while filter feeders did not. In spite of the ability of krill to graze this alga, it

appears that diatoms make up most of the diet of krill in Antarctic waters (Meyer & El-Sayed 1983, Miller & Hampton 1989). Raptorial grazing by copepods on Phaeocystis was reported by Weisse (1983) who found that colonies between 50 and 350 μm diameter were preferred. Calanus finmarchicus, C. hyperboreus and Thysanoessa spp. grazed Phaeocystis colonies from 30 to 100 μm diameter at the same rate as diatoms and tended to ignore large colonies (Hansen et al. 1990, 1994). They also found that animals at later developmental stages preferred the larger food particles. Large suspension feeding copepods also appear capable of grazing Phaeocystis (Huntley et al. 1987, Tande & Båmstedt 1987) while Miller and Hampton (1989) suggest that this alga is avoided by most small metazooplankton. Larger colonies escaped predation by copepods (Gráneli et al. 1993). However, feeding and swimming activity of the copepods (Thysanoessa spp., Calanus hyperboreus and C. finmarchicus) is apparently responsible for fragmentation of colonies and release of solitary colonial cells (Hansen et al. 1994). The resulting changes in particle size change the availability Phaeocystis colonies to higher trophic levels.

Calanus finmarchicus and C. hyperboreus can both grow on a diet of Phaeocystis (Tande & Båmstedt 1987) as can Acartia clausi and Temora longicornis (Weisse 1983). However, Verity and Smayda (1989) found that grazing rates of Acartia hudsonica and A. tonsa on Phaeocystis flagellate cells and colonies were very low and that egg production was related to the concentration of diatoms alone. When offered only Phaeocystis, egg production of the copepods fell to the level of the starved controls. In a field study Claustre et al. (1990) found that, although Phaeocystis comprised 97% of the algal biomass, the diatoms comprised some 74% of the copepod diet.

Lancelot and Rousseau (1994) propose that colonisation of decaying colonies by attached auto- and heterotrophic communities could considerably alter the nutritional quality of Phaeocystis colonies. Enhanced food quality as a result of such colonisation may explain the findings of Estep et al. (1990) that predation rate on Phaeocystis colonies changed as a function of their physiological state, increasing markedly during senescence. However, the

ability of colonies to bud and cleave (see section 3.3.3) escapes such microbial colonisation and may avoid grazing of potentially susceptible free living cells (Lancelot & Rousseau 1994). Initiation of flagellate release upon the decline of Phaeocystis blooms (see section 3.2.4) would also reduce cell loss by sedimentation and grazing of senescent colonies. The growth rate of flagellate cells is apparently higher than the colonial life stage (Baumann et al. 1994). At the inception of blooms rapid growth and proliferation of flagellate cells may provide the initial buildup of Phaeocystis concentration. By forming colonies, these free-living cells may escape being grazed (Verity et al. 1991).

5.4. Microheterotrophs

Microheterotrophs appear capable of grazing Phaeocystis (Hollowday 1949, Fryxell et al. 1984, Admiraal & Venekamp 1986, Lutter et al. 1989, Wassmann et al. 1990, Weisse & Scheffel-Möser 1990, Davidson & Marchant 1992a, Hansen & van Boekel 1991, Hansen et al. 1993). Ciliates, heterotrophic dinoflagellates and choanoflagellates are well suited to rapid population increase in response to the spectacularly rapid development of Phaeocystis blooms and Admiraal and Venekamp (1986) suggested that tintinnid grazing was sufficient to limit the duration of its bloom. Grazing of solitary Phaeocystis cells by protozoa reportedly greatly exceeds that by the copepod Temora longicornis (Hansen et al. 1993). However, T. longicornis grazes protozoa. The resulting decline in protozoan concentration reduces grazing pressure on Phaeocystis cells by 21 and 67% (Hansen et al. 1993). Thus, metazooplankton may enhance the relative abundance of Phaeocystis by preferentially grazing co-occurring phytoplankton species (Verity & Smayda 1989, Claustre et al. 1990) and by consuming organisms that graze Phaeocystis. Furthermore, utilisation of Phaeocystis blooms by microheterotrophs and the "microbial loop" may form an important link with higher trophic levels (Davidson & Marchant 1992a, Hansen & van Boekel 1991, van Boekel et al. 1992, Fernández et al. 1992, Hansen et al. 1993, Thingstad & Billen 1994, Weisse et al. 1994).

In summary, proto- and metazooplankton will graze Phaeocystis (Table 2). The extent and rate of their grazing is limited by feeding behaviour and the structure and geometry of their feeding apparatus. These change with the developmental stage of a grazer and determine significantly the particle size available to it. The structural limitation of particle size available to a grazer is of particular importance when considering Phaeocystis because of the enormous potential range in size of this alga. However, zooplankton capable of consuming Phaeocystis colonies over a particular size range will do so (Table 2). Thus, particle size alone may largely determine zooplankton grazing pressure on Phaeocystis blooms. Senescent decline of Phaeocystis colonies also reportedly ameliorates proposed chemical and nutritional deterrents to grazers. Clogging of feeding appendages (Martens 1981, Schnack 1983) is likely to effect those organisms using specific modes of feeding. Fragmentation of Phaeocystis colonies by swimming and feeding activity (Hansen et al. 1994) or other agents of mechanical disruption may release solitary colonial cells from the colony matrix and avoid clogging of the feeding apparatus.

5.5. The fate of Phaeocystis blooms

Selective avoidance of Phaeocystis by grazers may contribute to the development of its blooms (Verity & Smayda 1989). Holligan (1987) and Paerl (1988) point out that it is often species like Phaeocystis and Gyrodinium aureolum, which are avoided by grazers, that form exceptional blooms. Claustre et al. (1990) found only 1.5% of the biomass of a Phaeocystis bloom was grazed by copepods, the remainder apparently being lost to the pelagic food web. The unique physiology of Phaeocystis and growth of this alga relatively unconstrained by grazing mortality results in massive accumulations of organic carbon and nitrogen (Bölter & Dawson 1982, Davidson & Marchant 1992a) which sediment within the euphotic zone to give the highest POC and PON sedimentation rates ever recorded (Wassmann et al. 1990). However, its blooms apparently contribute little to direct carbon flux to the deep ocean as much of the carbon fixed by this alga is respired by microheterotrophs and bacteria in the upper 100 m (Wassmann et al. 1990, 1991, Davidson

Table 2. Trophic and avoidance responses of organisms to Phaeocystis. Physical avoidance refers to organisms that avoid waters containing Phaeocystis blooms. Preferential avoidance refers to organisms that avoid grazing Phaeocystis but graze co-occurring phytoplankton species. Abbreviations: Acrylic acid (AA), dimethylsulfide (DMS), particulate and dissolved organic carbon (POC and DOC respectively) and NA means not applicable.

Organisms	Feeding Mode	Avoidance	Proposed Characteristics and Processes	Effect	Amelioration of Effect	Recorded Grazers of Phaeocystis	Proposed Reason for Grazing
Fish	Do not graze	Physical	Chemically and physically deter (AA, DMS and anoxia), mucilage	Clogging of gills	NA	NA	NA
Shellfish	Ciliary	Preferential	Mucilage cloggs feeding appendages	Starvation	-	Yes	
Benthic infauna (Rogers and Lockwood (1990)	No report of grazing	Unable to avoid	Sedimented anoxic bloom	Asphyxiation	-	-	
Protozoa	Ciliary, pseudopodial, filter	No	NA	NA	Senescent colonisation by bacteria and build-up of POC and DOC	Yes	Rapid division rates and abundant carbon substrate
Metazooplankton (Euphausiids, copepods, cladocerans, meroplanktonic larvae)	Filter and raptorial feeding, selective grazers	Preferential	Poor nutritional quality	Reduced growth, egg production	Senescent colonisation by bacteria and microheterotrophs	Yes	Increased nutritional quality
			Chemically deter (AA and DMS)	Not toxic	Senescent physiological state	Yes	Not deterrent
		Physical	Trophic mismatch	Sub-optimal grazing	NA	Yes	
			Mucilage cloggs feeding appendages	Inefficient grazing	Disruption of colony	Yes	Releases solitary colonial cells from mucilage
			Particle size of colonies	Escapes predation	Swimming and feeding-induced colony fragmentation	Yes	Particles within structural limits of feeding apparatus

& Marchant 1992a, Riebesell 1993, Wassmann 1994, Passow & Wassmann 1994, Brussaard et al. 1995). Avoidance of Phaeocystis by grazing zooplankton would mean that this alga also contributes little to vertical carbon flux as faeces and moulted exoskeletons (Marchant & Davidson 1991). Thus, despite accounting for a significant proportion of the primary production in the higher latitudes, the poor pelagic to benthic coupling of Phaeocystis blooms means that it is unlikely to play an important role in sequestration of carbon from the atmosphere (Wassmann 1994).

Some authors report that Phaeocystis spp. colonies are positively buoyant (Skreslet 1988, Reibesell 1993). While aggregate formation as a result of turbulent mixing may result in accelerated sinking rates, the potential for Phaeocystis to form aggregates is apparently low in comparison with diatoms (Reibesell 1993). In contrast, Wassmann et al. (1991) observed very rapid sinking of a Phaeocystis bloom in the Barents Sea. This alga also has been found to contribute to carbon flux to deep Atlantic waters as a result of subduction of its blooms beneath Arctic surface water (Smith et al. 1991). Thus, Phaeocystis spp. blooms may contribute to deep carbon flux under specific conditions. The physical conditions responsible for the rapid sinking observed by Wassman et al. (1991). However, repeated observations that Phaeocystis blooms sink slowly (eg. Wassmann 1994) suggest that rapid sinking is uncharacteristic.

6. PHYSIOLOGY

Phaeocystis possesses a peculiar physiology (Lancelot et al. 1987) and it is in the colonial stage that these peculiarities are exhibited, enabling it to outcompete other algae. Despite the importance and ubiquity of Phaeocystis, its physiology, growth and metabolism are not well understood.

6.1. Growth

The factors that determine the growth rate of Phaeocystis are the subject of some conjecture. It has been found to depend on inorganic nutrient availability, irradiance,

and/or temperature (Bätje & Michaelis 1986, Weisse et al. 1986, Lancelot & Mathot 1987, Verity et al. 1988a). Lancelot and Mathot (1987) found that photosynthetic rate, mucilage production, and thus to some extent increase in colony diameter, are independent of both ambient nutrient concentration and temperature. However, Verity et al. (1988a) indicated that irradiance and nitrate concentration appear to be the two major determinants of growth and photosynthetic rates in Phaeocystis.

In the only report describing the genesis of a Phaeocystis bloom, Bätje and Michaelis (1986) reported isolated patches of red-brown discolouration which increase in diameter and spread over the entire area. Thus, the cells necessary as a seed source for the bloom as well as the conditions conducive to their proliferation occur simultaneously and independently over a considerable area. This gives rise to patches of Phaeocystis which coalesce during development of the bloom. Its outbursts have been attributed to various physical and biotic factors (see Section 6.3, 6.4) however, the factor/s initiating blooms are unclear (Cadée & Hegemann 1986, Weisse et al. 1986, Lancelot et al. 1987).

Weisse et al. (1986) reported that Phaeocystis blooms occur between the spring diatom bloom and the development of the summer phytoplankton assemblage in temperate latitudes. They concluded that Phaeocystis does not replace other species but instead provides extra production to the system. This is not true of polar waters where Phaeocystis commonly precedes the diatom bloom (Gran 1929, 1930, Smayda 1980, Davidson & Marchant 1992a) and seems only partially sustainable in temperate latitudes where Phaeocystis is commonly observed to have an antagonistic effect on co-occurring phytoplankton. Proliferation of Phaeocystis inhibits the development of populations of other phytoplankton groups (Lucas 1940, Jones & Haq 1963, Smayda 1973, Barnard et al. 1984, Admiraal & Venekamp 1986, Bätje & Michaelis 1986, Veldhuis et al. 1986b, Weisse et al. 1986, Davidson & Marchant 1992a). Thus, it is equally possible that the bloom of Phaeocystis interrupts or terminates the diatom bloom. Possible reasons for this limitation of other algae include preferential grazing by copepods on diatoms (eg. Verity

& Smayda 1989, Claustre et al. 1990), the ability of Phaeocystis to outcompete other phytoplankton for macronutrients (Laandbroek et al. 1985), silicate depletion by diatoms (Verity et al. 1988a) and accumulation of trace metals by Phaeocystis (Davidson & Marchant 1987, Lubbers et al. 1990). These are discussed in more detail in Section 6.3.

6.2. Temperature

Phaeocystis grows in a wide variety of environments. Kashkin (1963) characterised this species by as eurythermal and reports indicate that Phaeocystis isolated from different thermal environments have different temperature tolerances (Table 3). Growth rates presented are merely indicative as they are not maximum growth rates; these also vary between strains. Originally, Phaeocystis was thought to have developed many thermally distinct strains. The extent of the thermal range, though still extensive, has been reduced by the taxonomic resurrection of P. antarctica and P. globosa. Baumann et al. (1994) attempt to define the thermal limits of the Phaeocystis species now recognised. P. antarctica tolerates temperatures down to -2°C and Baumann et al. (1994) state that the upper temperature tolerated is 2°C. However, isolates of P. antarctica from Prydz Bay, Antarctica, maintain healthy growth at 4°C (Marchant et al. 1991). Thus, the upper limit of temperature tolerance of this species remains unknown. P. pouchetii reportedly tolerates temperatures between -2 and 14°C and P. globosa tolerates temperatures between 0 - 22°C (Baumann et al. 1994), though differences exist between isolates. The taxonomic identity and temperature tolerance of Phaeocystis reported from equatorial waters is unknown but it survives temperatures in excess of 36°C (Al-Hassan et al. 1990).

The range of temperature occupied by each species suggests that no absolute temperature can be proposed for bloom initiation throughout its range (Jones & Haq 1963, Weisse et al. 1986). Thus it appears that no one temperature initiates Phaeocystis blooms at a single site (Cadée & Hegeman 1986, Weisse et al. 1986). Strains of differing thermal tolerance would be expected to respond differently to temperature change. Thus, it may be the rate

or magnitude of the change in temperature that elicits a physiological response from the alga (Verity et al. 1988a).

Table 3. The temperature tolerance and growth rates of different strains of Phaeocystis isolates from tropical, temperate and polar waters.

Temperature at which growth rate calculated (°C)	Temperature range of growth (°C)	Growth rate (Doublings / Day)	Author
2-6	2-12	0.16-0.8	Verity et al. 1988a
3-6	Not Given	0.4-1.1	Nøst-Hegseth 1982
4	Not Given	0.22	Marchant et al. 1991
6	≤4-13	0.8-1.3	Guillard & Hellebust 1971
15	5-≥18	Not Given	Kayser 1970
15	7-20	3.4	Grimm & Weisse 1985
20	17≥27	1.5-2.0	Guillard & Hellebust 1971

6.3. Nutrients

The concentrations of nitrate (Bougard 1979, Eilertsen & Taasen 1984, Bätje & Michaelis 1986, Reigman et al. 1990) or phosphate (Jones & Haq 1963, van Bennekom et al. 1975, Gieskes & Kraay 1975, Veldhuis et al. 1986b) have been suggested as significant determinants of the timing, extent and duration of the Phaeocystis bloom. This alga is reportedly tolerant of low concentrations of phosphate (Weisse et al. 1986) which may be due to its reported ability to store phosphate in the colonial stage (Veldhuis & Admiraal 1987). Unlike the flagellate stage, uptake of phosphate by colonial cells is maintained in the dark and the energy requirements for this dark assimilation are met by catabolism of intracolonial carbon (see Section 6.5) (Veldhuis et al. 1991). However, Cariou et al. (1994)

found that phosphate concentrations between 0.3 and 5 μM were required to support the colonial stage (see section 3.2.4). This apparent obligate requirement for moderate phosphate concentration by the colonial stage of Phaeocystis is supported by its ability to utilise organic phosphate substrates by alkaline phosphatase activity (APA) (van Boekel & Veldhuis 1990). APA hydrolyses the P-O-C bonds of organic phosphates, releasing inorganic phosphate for uptake by the cells. Many organic phosphate substrates supported equivalent growth of P. pouchetii to that of inorganic phosphate (van Boekel 1991). Thus, APA enhances phosphate availability to P. pouchetii colonies and may aid their persistence. However, APA synthesis decreased at inorganic phosphate concentrations below 0.5μM (van Boekel & Veldhuis 1990) indicating that APA may do little to ameliorate phosphate limitation under conditions of severe inorganic phosphate depletion. APA also did not increase under elevated organic phosphate concentrations (van Boekel & Veldhuis 1990). High organic phosphate concentrations would therefore be under-utilised.

Phaeocystis appears adept at growing under conditions of low nitrate concentration (Verity et al. 1988a). The colonial stage of this alga predominates in nitrogen controlled environments (see section 3.2.4). While providing both a store of nutrient and carbon substrate, the mucilaginous envelope of the colony does not greatly impede nutrient uptake rates (Veldhuis et al. 1987, 1991). Jahnke (1989) contends that Phaeocystis has a relatively poor capacity to store phosphate and indicates that it would not provide a competitive advantage over diatoms. However, the nutrient uptake dynamics reported by Veldhuis et al. (1991) may provide an advantage over organisms limited to nutrient assimilation during exposure to light. In contrast, the proposed tolerance of low macronutrient concentration does not explain the almost exclusive occurrence of flagellate Phaeocystis in oligotrophic waters (Estep et al. 1984) and nutrient enrichment resulting in the appearance of the colonial stage in tropical waters (Al-Hassan et al. 1990).

The observed capacity of Phaeocystis to thrive at low macronutrient concentration (Jones & Haq 1963, Bougard 1979, Eilertsen & Taasen 1984) appears advantageous to the

species in temperate latitudes where its blooms occur after the diatom bloom (eg. Eberlein et al. 1985, Bätje & Michaelis 1986, Veldhuis et al. 1986b, Weisse et al. 1986, Veldhuis et al. 1988). Apparently, nitrate in particular remains in the water column following the diatom bloom (Bätje & Michaelis 1986, Weisse et al. 1986) and this nutrient may well determine the magnitude of the Phaeocystis bloom (Bougard 1979, Eilertsen & Taasen 1984, Lancelot et al. 1986, Verity et al. 1988a, Colijn et al. 1990). Data from El-Sayed et al. (1983) indicate that P. antarctica blooms result in significantly greater depletion of nitrate than other phytoplankton species. Most authors (Jones & Haq 1963, van Bennekom et al. 1975, Gieskes & Kraay 1975, Cadée & Hegeman 1986, Weisse et al. 1986, Veldhuis et al. 1986b, Veldhuis 1987) agree that phosphate depletion eventually limits the Phaeocystis bloom, others (Lancelot 1983, Lancelot 1984b, Lancelot & Billen 1984, Lancelot & Mathot 1985) however, reported nitrogen as being the limiting nutrient.

In contrast to diatoms, Phaeocystis has no nutritional requirement for silicate (eg. Codispoti et al. 1990, Stefánsson & Ólafsson 1990). This may account for the appearance of Phaeocystis blooms after diatoms in the temperate latitudes. Diatoms exhaust the available silicate during their blooms and several authors (Jones & Haq 1963, Jones & Spencer 1970, van Bennekom et al. 1975, Gieskes & Kraay 1975, Cadée & Hegeman 1979, 1986, Colijn 1983, Veldhuis et al. 1986b, Weisse et al. 1986, Veldhuis & Admiraal 1987, Verity et al. 1988a) propose that this allows Phaeocystis to utilise the remaining macronutrients. Verity et al. (1988a) also showed that in mixed phytoplankton communities, replenishment of silicate results in co-occurrence of Phaeocystis and diatoms. In contrast, Laandbroek et al. (1985) reported that silicate remains in the water column during the Phaeocystis bloom and declined after its collapse. The fact that silicate remained unused by diatoms during the Phaeocystis bloom was considered evidence that Phaeocystis outcompetes diatoms for nutrients (Laandbroek et al. 1985).

No mechanism has been proposed to determine the position of Phaeocystis in the phytoplankton species succession in polar waters. Unlike cool temperate and sub-polar

waters, such as the North Sea and English Channel, blooms of Phaeocystis do not develop following limitation of diatom abundance by silicate depletion. In polar waters Phaeocystis is the first species to bloom (see Section 4). It remains to be seen what causes this fundamental difference in its behaviour in polar waters.

The lack of dependence by Phaeocystis on silicate concentration may be important in determining its proliferation in coastal waters of the North Sea. Agricultural runoff and domestic waste have enriched these waters with nitrate and phosphate but not silicate. This selectively advantages flagellates at the expense of diatoms (Owens et al. 1989) and at least some strains of Phaeocystis appear well suited to proliferating in these eutrophic conditions (Lancelot et al. 1987). Nutrient enrichment increases the concentration and duration of blooms of this alga (eg. Lancelot & Billen 1984, Cadée & Hegeman 1986, Lancelot et al. 1987). Thus, Phaeocystis can proliferate in a broad spectrum of nutrient environments from diatom depleted concentrations to the eutrophic levels encountered in Northern European coastal waters (Veldhuis et al. 1987).

Nutrient stress has been observed in Phaeocystis. At low concentrations it causes increased carbon to chlorophyll *a*, nitrogen and ATP ratios and, under conditions of chronic deprivation, results in the alga assuming the flagellate form (Verity et al. 1988b). Low phosphate concentrations also lead to increased carbon excretion rates (Veldhuis et al. 1986a), an increase in alkaline phosphatase activity (Admiraal & Veldhuis 1987, Veldhuis & Admiraal 1987) and usage of enzymatically hydrolysable phosphorus (Veldhuis et al. 1987). Low nitrate concentration leads to decreased protein synthesis, increased production of polysaccharides (Lancelot et al. 1986), and a greater proportion of production being expended on secretion of mucilage than metabolism (Lancelot 1983, Lancelot & Mathot 1987). Such metabolic effects of nutrient deprivation may exclude Phaeocystis from blooming in oligotrophic tropical waters.

Highly nutrient enriched culture media have also been reported as increasing proportion of flagellates in cultured Phaeocystis (Kayser 1970, Veldhuis & Admiraal 1985, 1987)

and data from Bätje and Michaelis (1986) and Cadée and Hegeman (1986) suggest that the number of flagellate blooms in eutrophic waters of the coastal northern Europe has increased (Owens et al. 1989). Antarctic waters are rich in macronutrients (Jacques 1983, Holm-Hansen et al. 1977). However, blooms of Phaeocystis from these waters reportedly consist almost entirely of the colonial stage (eg. Buck & Garrison 1983, Fryxell & Kendrick 1988, Davidson & Marchant 1992a). Changes observed in the concentration of the flagellate life stage during the Phaeocystis bloom, are likely to reflect changes in life stage rather than being related to nutrient concentration (Davidson & Marchant 1992a). Colonial and flagellate cells have been observed at all nutrient concentrations that would support cell growth (Veldhuis & Admiraal 1987, Verity et al. 1988a). Thus, unlike temperature changes (Verity et al. 1988b), change in nutrient concentration did not elicit a change in cell stage by the entire Phaeocystis population. Instead it caused a shift in the ratio of flagellate to colonial cells (Kayser 1970, Veldhuis & Admiraal 1985). Verity et al. (1988b) proposed that such changes may also be determined by endogenous factors such as the possible development of sexuality, or behavioural factors including escape from conditions that are stressful to the colonial stage.

Phaeocystis has been shown to accumulate trace metals (Morris 1971), particularly manganese (Davidson & Marchant 1987, Lubbers et al. 1990). This accumulation of up to 75% of the available soluble manganese is caused by photosynthetic CO_2 uptake and O_2 evolution which increases the pH and Eh within the microenvironment of the colony (Lubbers et al. 1990). The increased pH results in oxidation of the manganese to an insoluble brown precipitate and Davidson and Marchant (1987) reported that it is the presence of these oxides in the colony matrix that accounts for the characteristic colouration of Phaeocystis blooms (eg. Savage 1930, El-Sayed et al. 1983). Colonial Phaeocystis also has a demonstrated bacteriocidal capacity (see section 6.9) which would result in limited remineralization of the accumulated manganese. Thus, by accumulating this micronutrient which is essential to plant growth (O'Kelly 1974) and limiting its re-

release this alga may mediate the phytoplankton species succession (Davidson & Marchant 1987, Lubbers et al. 1990).

Although the availability of nitrogen and phosphorus clearly effects the onset of Phaeocystis blooms, there are no absolute concentrations that trigger their onset (Table 4). They are a poor determinant of the relative proportions of each stage in the life cycle of natural blooms of this species. Thus, life stage and bloom development by this species is apparently mediated by a number of environmental factors, one of which is macronutrient concentration.

Mixotrophy has been proposed for this alga (Chu 1946, Kornmann 1955, Jones & Haq 1963, Foster et al. 1983, Weisse et al. 1986) but not demonstrated. Weisse et al. (1986) suggested utilisation of diatom ectocrines by Phaeocystis while Boalch (1984) found that formation of colonies from the flagellate stage of Phaeocystis was enhanced by chemical products of Chaetoceros. Weisse et al. (1986) also postulated the converse, namely that ectocrines from blooms preceding Phaeocystis were inhibitory to its development. No empirical data have been provided to support such an interaction and these suggestions perhaps better reflect the lack of understanding of Phaeocystis bloom initiation than any real mixotrophic activity. Addition of soil extract to cultures reportedly enhances growth of Phaeocystis and it has been suggested that this represents mixotrophic utilisation of terrigenous compounds (Chu 1946, Kornmann 1955, Jones & Haq 1963). In contrast, Kayser (1970) found that Phaeocystis grew poorly in culture media containing soil extract. Such differences probably reflect the variability in soil quality but may be due to other differences in the culture medium.

Our observations indicate Phaeocystis antarctica does grow well in media containing soil extract. However, no mixotrophic nutrient sources are obligatory as the colonial stage

Table 4. Effect of macronutrient concentration on the physiology and life cycle of Phaeocystis.

	Nitrogen	Phosphorus
Intracolonial Accumulation	Yes	Yes
Low Concentration	Tolerant	Tolerant
Effect at Low Concentration	1. Decreased protein synthesis 2. Decreased polysaccharide production 3. Increased proportion of production as mucilage	1. Increased carbon excretion rates 2. Increased alkaline phosphatase activity 3. Use of enzymatically hydrolysable phosphate
Limits Duration & Extent of Blooms	Yes	Yes
In Culture		
Colonial Stage Favoured	Low Concentration	Low Concentration
Flagellate Stage Favoured	High Concentration	High Concentration
In situ		
Colonial Stage Favoured	1. N controlled eutrophic northern european waters 2. High N polar waters 3. Eutrophic Tropical waters	1. High P:N ratio eutrophic northern european waters 2. High P polar waters 3. Eutrophic Tropical waters
Flagellate Stage Favoured	1. Low N oligotrophic warm temperate & tropical waters 2. High N in eutrophic northern european waters	1. Low P oligotrophic warm temperate & tropical waters 2. High P in eutrophic northern european waters

was successfully maintained over more than six culture generations in Aquil synthetic sea-water (Morel et al. 1979) from which soil extract and vitamins were omitted (Davidson unpubl.). Experiments using 1.97 and 0.21 micron diameter, protein-coated flourescently labelled microspheres and 9400 molecular weight fluorescent dextrans (after Marchant & Scott 1993) showed no evidence of uptake by Phaeocystis motiles after incubation for 1 day at 2°C in the dark (Davidson unpubl.).

6.4. Light

Reported responses of Phaeocystis to light intensity vary. Verity et al. (1988a) claim that it is able to utilise light at higher irradiances than other algae. This is consistent with reports of Phaeocystis being commonly observed in surface waters. Again, however, this alga exhibits extraordinary environmental plasticity. It is reported to adapt to light climates ranging from 1600 $\mu Em^{-2}s^{-1}$ (Palmissano & Sullivan 1985) for P. antarctica to 16 $\mu Em^{-2}s^{-1}$ (Palmissano et al. 1986). Eilertsen (1989) and Joint and Pomroy (1981) report high photosynthetic efficiency of both the colonial and flagellate Phaeocystis at low light intensities. Palmissano et al. (1986) showed that P. antarctica adapted to the low-light conditions beneath sea ice by increasing its photosynthetic efficiency three to four fold per unit chlorophyll a and two to three fold per cell. This adaptation reportedly involved increasing its absorption of blue-green wavelengths (SooHoo et al. 1987). While able to adapt to low light intensities, saturating light intensities for Phaeocystis are reportedly high (Colijn 1983). Lancelot and Mathot (1987) found that low light adapted Phaeocystis suffered no significant light inhibition at high intensities and they proposed that this may be due to attenuation of light by the mucilaginous envelope. However, this is unlikely as Marchant et al. (1991) showed that the wavelengths absorbed by mucilage are too short for it to afford significant protection against solar radiation.

The colonial stage of P. antarctica strongly attenuated ecologically significant light wavelengths below 370 nm (Marchant et al. 1991) irrespective of past UV climate.

Production of UV-absorbing compounds could, however, be further enhanced by irradiation with UV-B light. Possession of these compounds by the colonial stage provides substantial protection against damage by UV-B radiation, however, the flagellate stage lacks the pigmentation and temperate, tropical and northern hemisphere colonial strains of Phaeocystis contain 5 to 10 times less of these absorbing compounds and are correspondingly less able to survive UV-B exposure (Marchant et al. 1991).

The ice-edge bloom provides much of the production in the Southern Ocean (Smith & Nelson 1986) which is a major nutrient source to sustain the abundant life at higher trophic levels. P. antarctica is a significant contributor to these blooms (eg. SooHoo et al. 1987, Garrison et al. 1987, Fryxell & Kendrick 1988) and producing UVB-absorbing compounds is consistent with its occupancy of these near surface waters. However, spring-time UV-B irradiances are increasing due to stratospheric ozone depletion (Stolarski et al. 1986) and these enhanced UVB irradiances coincide with the ice-edge bloom. This led Marchant and Davidson (1991) to propose that P. antarctica could become increasingly dominant at the expense of diatoms in these waters. In contrast, Smith et al. (1992) showed that growth of P. antarctica was significantly decreased by exposure to UVB radiation. Karentz and Spero (1995) found that the concentration of P. antarctica in the Bellinghausen Sea was positively correlated with changes in ozone and $\delta^{13}C$ of seawater ΣCO_2; and concluded that P. antarctica is rapidly and adversely effected by UVB exposure. However, the physiological state of cells in the study by Smith et al. (1992) is unknown and the study by Karentz and Spero (1995) did not measure other possible causes for P. antarctica mortality such as colony disruption or grazing.

Competition experiments were conducted using P. antarctica mixed with selected species of Antarctic diatoms and exposed to natural Antarctic solar irradiance (Davidson et al. in press). These showed that P. antarctica dominated at the expense of diatoms in treatments exposed to UVB wavelengths. This could profoundly effect the food web function,

nutritional status and production of higher trophic levels (Section 5.). In addition, P. antarctica appears to contribute less than diatoms to the direct vertical flux of carbon to deep water and the sea floor (see section 5.5) and would contribute little to carbon flux as faeces and moulted exoskeletons as Phaeocystis is reportedly poorly linked to higher trophic levels (Claustre et al. 1990). This may significantly reduce the capacity of the Southern Ocean to act as a sink for atmospheric CO_2 (Marchant & Davidson 1991).

6.5. Biochemical Composition

The commonly used technique of filtration to separate particulate from dissolved organic matter and cellular from extracellular material results in loss of colony matrix and contents to the filtrate (Bölter & Dawson 1982, Lancelot 1984b, Veldhuis & Admiraal 1985, Veldhuis et al. 1986a, Lancelot & Mathot 1987, Verity & Smayda 1989, Rousseau et al. 1990, Davidson & Marchant 1992a). Thus, it is difficult to discriminate between P. pouchetii cell contents, colony contents and extracolonial excretion (Bölter & Dawson 1982, Eilertsen & Taasen 1984, Lancelot 1984b, Veldhuis & Admiraal 1985, Verity & Smayda 1989).

As discussed earlier (Section 3.2.3) the extent of extracellular release by P. pouchetii reportedly ranges from 5 - 80% of its photoassimilated carbon. In addition, the amount of extracellular release by a population also varies over time (Veldhuis et al. 1986a). The majority of the substances released are low molecular weight mucopolysaccharides (Guillard & Hellebust 1971, Lancelot & Mathot 1987, Fernández et al. 1992). These may comprise around 40% of the primary production and are mainly utilised as colony matrix (Lancelot 1984a, Lancelot & Mathot 1987). As a consequence colonies have high carbon: nitrogen and carbon: chlorophyll a ratios (Verity et al. 1991). The colony matrix forms a biofilm in which mutually dependent biological and chemical processes are occurring for the benefit of the aggregated biological entity (Lancelot & Rousseau 1994). For this energetic expense the colony acquires an environment sufficiently isolated from the surrounding medium in which a unique biochemical environment can be created (see section 3.2.3). The

colonial habit also limits its availability to grazers (see section 5) and provides buoyancy (Skreslet 1988). Gelation of the mucopolysaccharides that forms the colony matrix is apparently due to the formation of magnesium and calcium salt bridges between carboxylated and sulphated polysaccharide chains (van Boekel 1992). The structural resilience of the colony matrix decreases with increasing colony size. The mucilage gel of large colonies may no longer provide enough support to protect the internal liquid volume against physical disruption (van Boekel 1992). The resilience of the colony mat be further reduced by catabolism of the polysaccharide matrix at low light (van Boekel 1992).

High light intensities reportedly cause inhibition of mucilage secretion, suggesting that it may suppress one of the steps in mucilage production (Lancelot & Mathot 1987, Verity et al. 1991). Consequently, exposure to high light may explain why some cultures form colonies with high cell densities and little mucilage (Nichols et al. 1991). Jahnke (1989) also implicated temperature in the carbon balance of the cells as cultures exposed to a rise in temperature decreased in carbon content.

Early growth of the Phaeocystis bloom exhibits rapid photosynthesis and protein synthesis being greater than carbohydrate production. By the peak of the bloom, carbohydrate was the dominant end product of photosynthesis (Veldhuis et al. 1986a). Supporting these findings, Hickel (1984) found that the ratio of particulate nitrogen to carbon fell sharply during the Phaeocystis bloom. However, Verity et al. (1988a) showed that Phaeocystis, in spite of its prolific release of photoassimilated carbon, had a carbon to nitrogen ratio similar to that of other non-gelatinous phytoplankton. According to Eberlein et al. (1985) breakdown of colonies released large amounts of dissolved organic nitrogen. This is at variance with reports of low nitrogen content of colonies (Hickel 1984) and low nitrogen content of the mucilage (Billen & Fontigny 1987, Lancelot et al. 1987, Rousseau et al. 1994). Veldhuis et al. (1986a) proposed that change in metabolism toward carbohydrate synthesis may represent the formation of storage products for the colony. Such intracolonial storage of macromolecules and their

catabolism during dark periods have also been proposed (Lancelot & Mathot 1985, Veldhuis & Admiraal 1985). Thus, the excretion of macromolecules is more than a means of building the colony matrix, it also participates in the energy balance of the constituent cells. This extracellular but intracolonial storage of photosynthate for catabolism is a metabolic pathway which is facilitated by possession of a colonial phase and highlights the function of the colony as a biological entity rather than a simple aggregation of cells. Dark catabolism of colony mucilage has been proposed as a significant feature in allowing Phaeocystis antarctica to occupy low light environments (Kang & Fryxell 1993). However, recent study by Matrai et al. (1995) indicates that P. antarctica does not utilize newly photoassimilated carbon in dark catabolism. Such contrasts in the physiology of P. antarctica with northern hemisphere members of the genus may reflect species differences or the extended polar day length or differences experimental methods.

The colony matrix also acts as a store for trace metals and phosphate and accumulation of these essential nutrients within the colony is apparently reversible, thereby making the store available to the cells (van Boekel 1992). These characteristics of the colonial stage, together with other recorded benefits such as production and maintenance of a UV screen and bacteriocide production (see sections 6.4, 6.9) provide a competitive edge for the colonial life stage.

Despite the benefits of colonial metabolism, the growth rate of flagellate cells apparently exceeded that of the colonial stage (Baumann et al. 1994). This could reflect the metabolic expense of forming and maintaining the colony matrix, the matrix acting as a diffusion barrier (van Boekel 1992) or a deficiency in mucilaginous energy reserves metabolised by the cells (Baumann et al. 1994). The photosynthetic rate of the flagellate stage reportedly exceeds that of the colonial stage (Verity et al. 1991). Coupled with the smaller biomass of the flagellate stage this would allow faster growth.

Polyunsaturated fatty acids in Phaeocystis vary considerably in composition and quantity (Sargent et al. 1985, Al-Hassan et al. 1990, Claustre et al. 1990, Nichols et al. 1991).

This variation may reflect differences in strains or species. It may also result from the differing physiological strictures, such as maintaining membrane function with changing temperature and increasing unsaturation of fatty acids at lower temperatures (Al-Hassan et al. 1990, Claustre et al. 1990). Lipid synthesis accounts for a relatively constant 20% of carbon fixed (Lancelot 1984b), however, as discussed in Section 5.3, P. pouchetii is low in neutral lipids, polyunsaturated fatty acid and vitamin C. As a consequence this alga is characterised as being of low nutritional value to grazers (Claustre et al. 1990). Differences in the fatty acid and lipid composition between stages in the life cycle of this alga suggest that the flagellate is marginally more nutritious than the colonial stage (Nichols et al. 1991).

Protein may account for between 20 and 42% of the total photoassimilated carbon (Lancelot 1984b). The rate of protein synthesis is, however, variable and is dependent on light history (Lancelot et al. 1986) and nitrate concentration (Lancelot 1984b). While Phaeocystis blooms are associated with high DON concentration (Eberlein et al. 1985) they also coincide with the lowest seasonal concentrations of dissolved free amino acids (DFAA) (Laandbroek et al. 1985) and low concentrations of ammonia (Eberlein et al. 1985, Laandbroek et al. 1985). These authors proposed that the low concentrations of the latter could be caused by either antibiosis of Phaeocystis blooms resulting in decreased heterotrophic decomposition of the available DFAA or the increased ammonia uptake and regeneration by this alga.

One conceptual feature of research into Phaeocystis colonies that has emerged recently is the appreciation of the colony as an integral unit rather than being a collection of autonomously functioning cells (Lancelot & Mathot 1985, Veldhuis & Admiraal 1985, Lancelot et al. 1986, Veldhuis et al. 1986a, Verity et al. 1988a). The same is also true of the colonial Phaeocystis biomass and production. Biovolume and colonial carbon biomass (Rousseau et al. 1990) rather than cell number appear to provide a far better

indication of production by Phaeocystis blooms (Lancelot & Mathot 1985, Veldhuis & Admiraal 1985, Veldhuis et al. 1986a).

6.6. Photosynthetic pigments

The concentration of some minor components of the pigments array in strains of Phaeocystis from opposite hemispheres reportedly differ. Chlorophyll c3 is ubiquitous in P. pouchetii (Vesk & Jeffrey 1987), but the major carotenoid concentrations were found to vary between strains. Bjørnland et al. (1988) found that the main carotenoid pigments in tropical Phaeocystis were fucoxanthin, 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxyfucoxanthin. Fucoxanthin dominated in the northern hemisphere strains with trace quantities of 19'-acyloxyfucoxanthins (Claustre et al. 1990, Gieskes & Kraay 1986) while in the southern hemisphere the dominance of these pigments was reversed with 19'-hexanoyloxyfucoxanthin comprising the majority (Wright & Jeffrey 1987, Nichols et al. 1991). Bjørnland et al. (1988) claimed the presence of 19'-acyloxyfucoxanthins distinguished the tropical strain from other Phaeocystis strains and proposed that this is further evidence of Phaeocystis being composed of more than one species. However, these carotenoids do occur in other strains (Gieskes & Kraay 1986, Wright & Jeffrey 1987) and their differing abundance could be construed as changes in biochemistry with environment and/or strain. Furthermore, Bjørnland et al. (1988) proposed that the acyloxyfucoxanthins comprised a chemosystematic marker. These pigments also occur in Emiliania huxleyi, Pelagococcus subviridis, (Wright & Jeffrey 1987), Corymbellus aureus (Gieskes & Kraay 1986) and some dinoflagellate genera (Marchant & Wright unpubl.) and, not being confined to Phaeocystis, they are not definitive of the species. However, Phaeocystis has an antagonistic effect on blooms of other algae (Section 6.1) and other acyloxyfucoxanthin-containing algae are sparse in the Southern Ocean. Thus these pigments may be useful as markers when combined with microscopic examination.

The chlorophyllase activity also varies between strains/species of Phaeocystis. Percent conversion of chlorophyll a to chlorophylide a was five times greater in P. antarctica than

Phaeocystis isolated from the East Australian Current (Jeffrey & Hallegraeff 1987). The reported differences in pigment complement and chlorophyllase activity may be due to genetic differences at the population or species level but may equally be due to physiological differences imposed by the ambient environment.

6.7. Extracolonial release

Phaeocystis blooms are often associated with very high DOC concentrations (eg. Bölter & Dawson 1982, Eberlein et al. 1985, Davidson & Marchant 1992a). Extracolonial release of carbon has been referred to as the only "true excretion" by Phaeocystis (Verity et al. 1988a). Carbon released in this way constitutes around 2-14 % of the photoassimilated carbon in naturally occurring populations (Laandbroek et al. 1985, Veldhuis et al. 1986a, Lancelot & Mathot 1987) and cultures (Veldhuis & Admiraal 1985, Veldhuis et al. 1986a). Thus, extracolonial excretion accounts for a relatively small proportion of total production (Cadée 1982). The DOC responsible for the spectacular occurrences of sea foam at the end of Phaeocystis blooms in the coastal zone of northern Europe (Eberlein et al. 1985, Bätje & Michaelis 1986) appears likely to be derived from collapse and decay of ungrazed production by this alga (Cadée 1982, Verity et al. 1988a).

6.8. Dimethylsulfide production

A cellular product of marine microorganisms, dimethylsulfoniopropionate (DMSP), which is thought to have an osmoregulatory function but may also be used as a cryoprotectant, structural component of cells, a buoyancy aid or a bacteriocidal agent (Sieburth 1961, 1964, Barnard et al. 1984, Variavamurthy et al. 1985, Karsten et al. 1995), can be cleaved to form acrylic acid and dimethylsulfide (DMS) (Sieburth 1960) (Fig. 6). DMS and its precursor DMSP are ubiquitous in the biosphere and are a common product of algae (Green 1962, Lovelock et al. 1972, Barnard et al. 1984, Variavamurthy et al. 1985). Enzymatic cleavage of DMSP to form DMS occurs both intra- and extra- cellularly (Liss et al. 1994) and occurs when algae die, are exposed to air

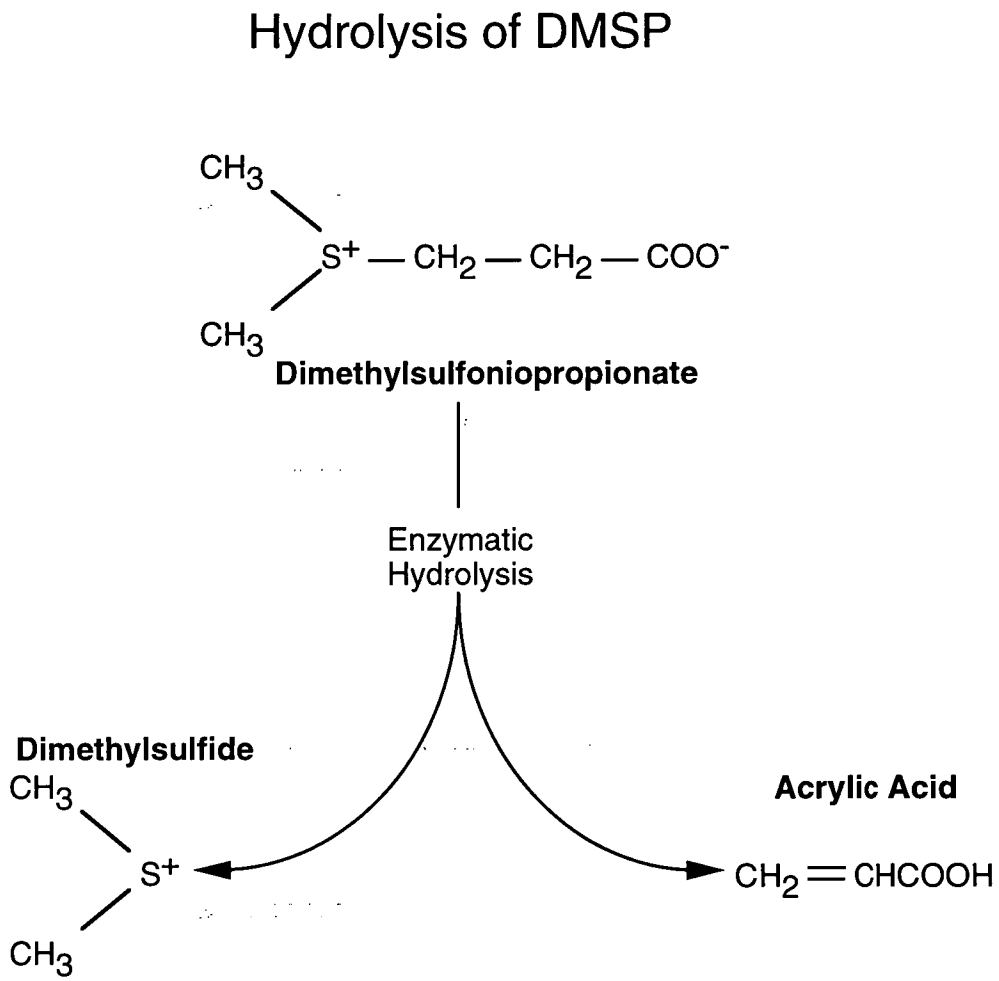


Fig. 6 The enzymatic hydrolysis of DMSP to form DMS and acrylic acid.

or as a consequence of normal metabolism (Barnard et al. 1984, Variavamurthy et al. 1985). Substantial release of DMS is restricted to a few classes of phytoplankton, mainly belonging to the Dinophyceae and Prymnesiophyceae (Keller et al. 1989). Such members of the Prymnesiophyceae as Hymenomonas carterae and Phaeocystis produce three orders of magnitude more DMS per cell than most other groups of phytoplankton (Barnard et al. 1984). DMS and DMSP production by Phaeocystis may represent as much as 20% its photoassimilated carbon (Matrai et al. 1995).

The abundance of Phaeocystis at higher latitudes correlates with greatly elevated concentration of this sulfur compound in the water column (Andreae & Raemdonck 1983, Barnard et al. 1984, Pearce 1988, Crocker et al. 1995), particularly in Antarctic waters (Deprez et al. 1986, Gibson et al. 1990). Phaeocystis reportedly possesses a very active DMSP-lyase enzyme which is specific to this alga (Stefels et al. 1995). Furthermore, cleavage of DMSP is apparently enhanced at low temperatures (Baumann et al. 1993) and high pH (Liss et al. 1994). High pH has been reported in Phaeocystis colonies (Davidson & Marchant 1987, Lubbers et al. 1990). The coincidence of an active DMSP-lyase, high colonial pH and cold temperatures at polar latitudes may contribute to Phaeocystis from Arctic and Antarctic waters being one of the most prolific producers of DMS. Most algal species release greatest quantities of DMS upon senescence (Kwint & Kramer 1995, Thingstad & Billen 1994). Stefels & van Boekel (1993) observed greatest release of DMS during exponential growth while other authors have observed highest concentrations of DMS (Matrai et al. 1995) and DMSP (Matrai et al. 1995, Liss et al. 1994) during the collapse of the bloom.

Biogenic DMS production may account for as much as 50% of the natural sulfur emission and 21% of the total global sulfur flux (Andreae & Raemdonck 1983). DMS is rapidly oxidised in the atmosphere to SO_2, methanesulfonate and sulfate (Hatakeyama et al. 1985, Yin et al. 1986). It is proposed that sulfate particles act as cloud condensation nuclei thereby establishing a mechanism for regulation of global albedo, and thus climate,

by marine biological activity (Charlson et al. 1987, Bates et al. 1987a) (Fig. 7). Additional ecological significance of these sulfate aerosols is evidenced by their 25-50% contribution to the total sulfur acids in Scandinavian air during North Sea phytoplankton blooms (Pearce 1988, Pain 1989).

DMS is also used by the microbial community. Microbial processes in the equatorial Pacific may remove DMS from the water column between 3 and 430 times faster than it is ventilated to the atmosphere (Keine & Bates 1990). During Phaeocystis blooms DMS production is between 1.5 and 4.5 times higher than the loss to the atmosphere (Stefels et al. 1995). Such differences between the proportion of DMS released to the atmosphere are dependent on the concentration, structure and function of the pelagic community including microbes, phytoplankton and grazers (Murray et al. 1992). Oxidation of DMS by bacteria and photochemical processes may lead to concentrations of DMSO exceeding those of DMS (Andreae 1980) and further oxidation of DMSO to sulphate would proceed around 500 times slower than for DMS (Brimblecombe & Shooter 1986). UV light is largely responsible for the photo-oxidation of DMS (Brimblecombe & Shooter 1986) and evidence of such photo-oxidation of DMS is reported by Crocker et al. (1995). During a Phaeocystis antarctica bloom in the Bellingshausen Sea, Antarctica, Crocker et al. (1995) observed lower concentrations of DMS in the upper 10 to 15 m of the water column despite P. antarctica exhibiting a surface maximum in abundance. These authors also observed diurnal changes in DMS concentration in surface waters. Maximum concentrations of around 125 nM DMS were observed at sunrise while minimum concentrations near 20 nM occurred around noon. The results indicate the potential for sunlight mediated changes in DMS concentration which may effect the flux of this compound to the atmosphere.

The world average DMS concentration in sea-water is approximately 2 nM (Bates et al. 1987b), however Gibson et al. (1990) measured concentrations as high as 290 nM during a near-shore bloom of Phaeocystis antarctica. Such extraordinarily high

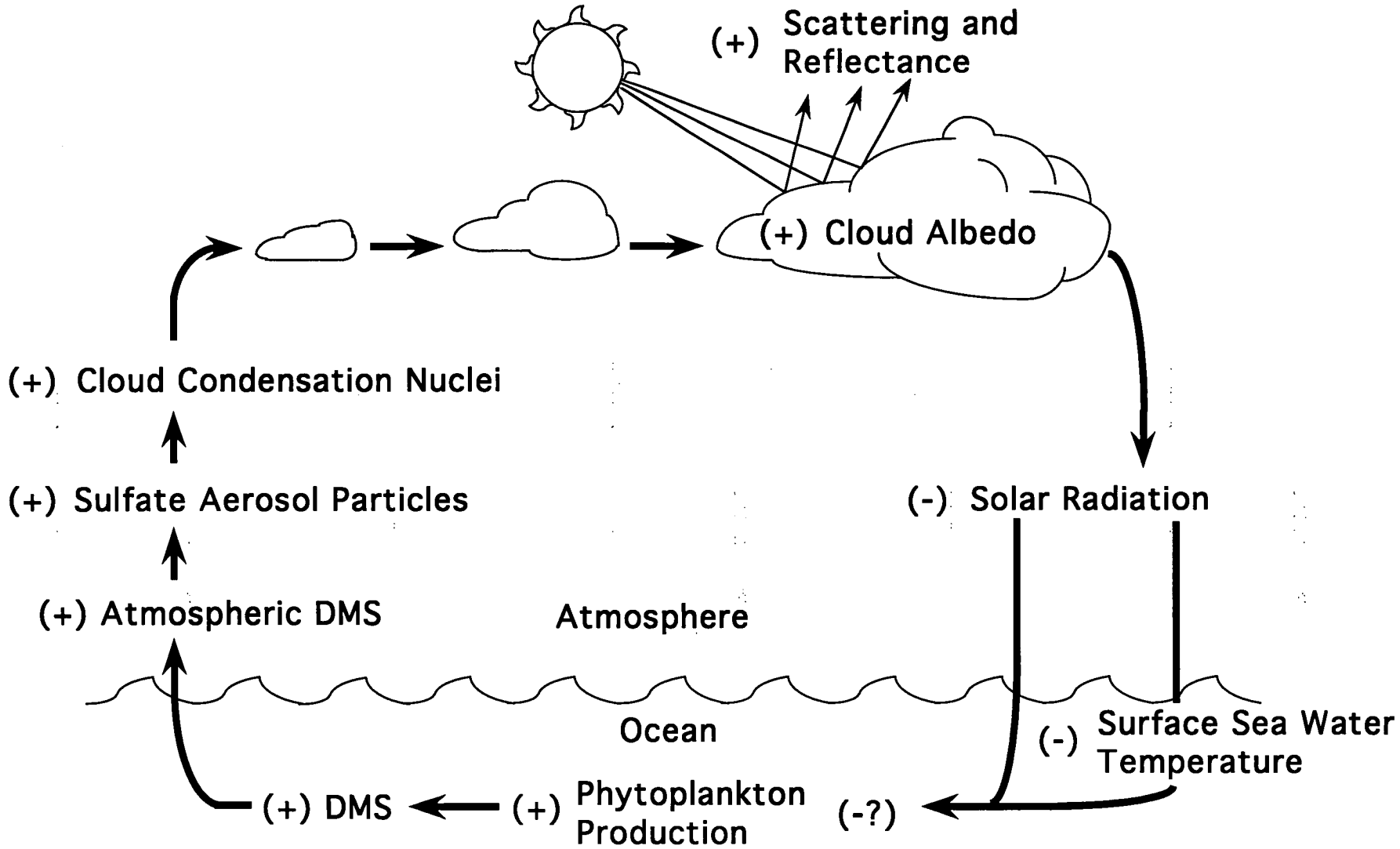


Fig. 7. Schematic diagram of the biogenic sulphur cycle.

concentrations of DMS in the water column led Gibson et al. (1990) to estimate that up to 10% of the global DMS flux to the atmosphere may emanate from Antarctic seas. Turner et al. (1995) claimed that DMS release from the Southern Ocean was currently uncertain and, although the flux rate they obtained for the region was similar to those previously published, may be up to 5.5 times higher than previously estimated, thereby increasing global DMS emissions by 25%. The rate of DMS production by Phaeocystis, its dominance of the water column and the widespread occurrence of this alga in high latitudes make it a major contributor to the global sulfur budget and therefore possibly able to influence global climate.

6.9. Acrylic acid and antibiosis

Photosynthetic products excreted by Phaeocystis to form the matrix and contents of its colonies are readily utilised by bacteria (Hickel 1982, Eberlein et al. 1985, Veldhuis et al. 1986b, Davidson & Marchant 1987, Verity et al. 1988a). However, these products largely avoid degradation in surface waters (Thingstad & Billen 1994). By enzymatically cleaving DMSP to produce DMS, Phaeocystis also release equimolar quantities of acrylic acid (Sieburth 1960) and this has been shown to inhibit bacteria (Sieburth 1960, 1961, 1979, Barnard et al. 1984). This is supported by the observation that colonies devoid of cells supported bacterial numbers two orders of magnitude greater than those containing cells (Verity et al. 1988b). Davidson and Marchant (1987) demonstrated bacterial inhibition in culture and showed that this bacterial defence was confined to the vicinity of each colony rather than throughout the entire culture. Eberlein et al. (1985) proposed suppression of bacterial growth by actively growing Phaeocystis as the reason for the accumulation of very high concentrations of organic carbon during its blooms and suggested that the proliferation of saprophytic bacteria in sea foam derived from Phaeocystis blooms (Eberlein et al. 1985, Gunkel 1982) represents loss of the alga's antibiosis upon the collapse of the bloom. Inhibition of bacteria would also result in the reduced decomposition of dissolved free amino acids and the low ammonia

concentrations observed by Eberlein et al. (1985). Antibiosis has been reported from field observations of P. antarctica blooms (Burkholder & Sieburth 1961, Davidson & Marchant 1992). Bacterial uptake rates reportedly increase during the bloom of Phaeocystis spp. but peaked as the bloom declined (Laandbroek et al. 1985, Lancelot & Billen 1984). This may reflect antibiosis during active growth of Phaeocystis spp. but may also be a temperature mediated lag between production and utilisation.

Some doubt surrounds the "antibiosis" of Phaeocystis colonies as a result of their acrylic acid production. Bacteria have been observed in close association with Phaeocystis blooms (Putt et al. 1994, Thingstad & Billen 1994) and acrylic acid concentrations reportedly seldom reach sufficient concentrations to inhibit bacterial production (Slezak et al. 1994). Billen and Fontigny (1987) claim close linkage between the bacterial biomass and substrate availability from a Phaeocystis bloom. Slezak et al. (1994) demonstrated that acrylic acid concentrations need to rise to 1 mM or above to inhibit bacterial production by 50% and it is claimed that, while healthy colonies of Phaeocystis are remarkably free of bacteria, these concentrations are not reached during Phaeocystis blooms (Thingstad & Billen 1994). In addition, Ledyard and Dacey (1990) suggest that, at the pH of sea water, low concentrations acrylic acid are an excellent substrate for bacterial growth. However, a bloom of Geminigera criophylla, a species not commonly regarded as a major DMSP producer, reportedly released concentrations of acrylic acid up to 2.47 mM in near-shore waters off Davis Station, Antarctica (Gibson JAE 1995 pers. comm.). Furthermore, such concentrations may regularly be reached in microzones within and surrounding Phaeocystis colonies or marine snow (Slezak et al. 1994). Thingstad and Billen (1994) did not consider such microzonal effects.

In excess of 80 - 90% of Phaeocystis colonies may be comprised of mucilage (Rousseau et al. 1990) and this material is apparently depauperate in nitrogen and phosphorus (Billen & Fontigny 1987, Lancelot et al. 1987). This nutrient deficiency may reduce the biodegradability of Phaeocystis material (Billen & Fontigny 1987, Lancelot et al. 1987,

Thingstad & Billen 1994). The proposed nutrient deficiency is supported by the observations of Rousseau et al. (1990) that mucilage synthesis is enhanced at low nutrient concentrations. However, Hickel (1984) refutes this claim, citing the observed proliferation of saprophytic bacteria in sea foam produced by Phaeocystis. Senescent colonies of Phaeocystis are also known to be heavily colonised by bacteria (Eberlein et al. 1985, Laandbroek et al. 1985, Veldhuis et al. 1986b, Davidson & Marchant 1987, 1991, Verity et al. 1988a, Thingstad & Martinussen 1991). Thingstad and Billen (1994) claim this does not reconcile with acrylic acid being used as a protectant against bacteria as production of DMS, and thus acrylic acid, is commonly at its highest during algal senescence. Matrai et al. (1995) reported that concentrations of DMS and DMSP in Phaeocystis colonies were highest during stationary phase and lowest during exponentially growth. However, Stefels and van Boekel (1993) found that DMS production by axenic cultures of Phaeocystis was highest during exponential growth and declined during stationary phase. Pelagic and epiphytic forms of bacteria reportedly proliferate throughout a Phaeocystis bloom (Putt et al. 1994) but the physiological state of the individual colonies supporting epibacteria in the study by Putt et al. (1994) remains unknown.

While published opinions concerning the effectiveness of the antibiosis vary, the bacterial defence mechanism proposed by Davidson and Marchant (1987) may reconcile these opposing views. Bacterial biomass and incorporation rates are dependent on the equilibrium between actively growing and senescent colonies at any stage in the population dynamics. Thus, bacterial numbers and incorporation rates may still be relatively high (Billen & Fontigny 1987) while allowing antibiosis of actively growing colonies. Thus, it appears likely that colonial Phaeocystis protects its energetic investment in the colony matrix against bacterial attack by producing acrylic acid and such protection allows the alga to benefit from the various attributes associated with this cell stage. If this is the case, as proposed by Barnard et al. (1984), the prolific release of DMS by this

alga, which has attracted much attention, is nothing more to the alga itself than a by-product of its protective mechanism.

6.10. Sea foam

A few days after the peak of a Phaeocystis bloom the colonies begin to break up. At this time characteristic development of sea foam is observed, forming long streaks on the open ocean (Eberlein et al. 1985, Rogers & Lockwood 1990). The foam results from dissolved organic carbon, composed of proteins and polymeric carbohydrates, released into the water column from decomposition of the massive POC generated during a bloom of Phaeocystis (Guillard & Hellebust 1971, Lancelot 1983, Veldhuis et al. 1986a). In contrast Eberlein et al. (1985) observed close correlation between Phaeocystis numbers and the concentration of DOM and concluded that the latter must result from direct exudation. The time of appearance of the sea foam would, however, indicate that the necessary concentrations of dissolved organics are reached only following collapse of the bloom. Blooms of Phaeocystis have been observed in the North Sea for a long time but the appearance of foam is a recent phenomenon, having first occurred in 1978 (Bätje & Michaelis 1986). This has been ascribed to the anthropogenic enrichment of northern European coastal waters (eg. van Bennekom et al. 1975, Reigmann et al. 1992) which in turn increases the frequency, abundance and duration of its blooms suggesting that strains of this alga are physiologically adapted to proliferation in nutrient enriched environments (Lancelot et al. 1987). Foam deposits reach depths of up to two metres on beaches in the area (Eberlein et al. 1985, Bätje & Michaelis 1986, Lancelot et al. 1987) being of substantial nuisance value (Lancelot et al. 1987). Harpacticoid copepods either passively or actively occupy the foam while appendicularians and nematodes are smothered by it (Armonies 1989).

7. CONCLUSION

Phaeocystis has become a nuisance alga in the North Sea (Lancelot et al. 1987) where the occurrence, abundance and duration of its blooms have increased (eg. Cadée & Hegeman 1986, Lancelot et al. 1987) in response to the anthropogenic enrichment of these coastal waters (eg. Bennekom et al. 1975). Hitherto, few occurrences of Phaeocystis have been reported from tropical waters, however, the appearance of blooms of this alga in the Arabian Gulf coincide with nutrient enrichment of these waters by sewage and industries (Al-Hassan et al. 1990). Based on its possession of UVB absorbing compounds Marchant and Davidson (1991) predict that this species could increase in dominance in Antarctic waters. Thus, it would appear that anthropogenic alteration of the environment enhances the abundance, dominance and distribution of Phaeocystis allowing them to display their prominence by forming essentially monospecific blooms. It remains to be seen whether future human alteration of the marine environment causes Phaeocystis increasingly to prosper at the expense of other phytoplankton. If this does occur, ecosystem structure and function in the areas where the species abounds will be strongly influenced by its unique physiology (Lancelot et al. 1987, Davidson & Marchant 1992a), potentially causing reduced secondary production (eg. Claustre et al. 1990), reduced vertical carbon flux (Marchant & Davidson 1991) and causing changes in the composition of food webs (Lancelot et al 1987, Davidson & Marchant 1992a) (Fig. 8).

Members of the genus Phaeocystis continue to inspire increasing scientific curiosity. The exponential increase in the number of publications over the last hundred years referring to Phaeocystis attest to its importance and fascination to biologists from many disciplines (Fig 9). Results of the research continue to reveal the complex physiology, life history, taxonomy and interaction with the biotic and abiotic environment. Intriguingly, published results are sometimes contradictory and while much is now known about Phaeocystis, much remains to be learnt.

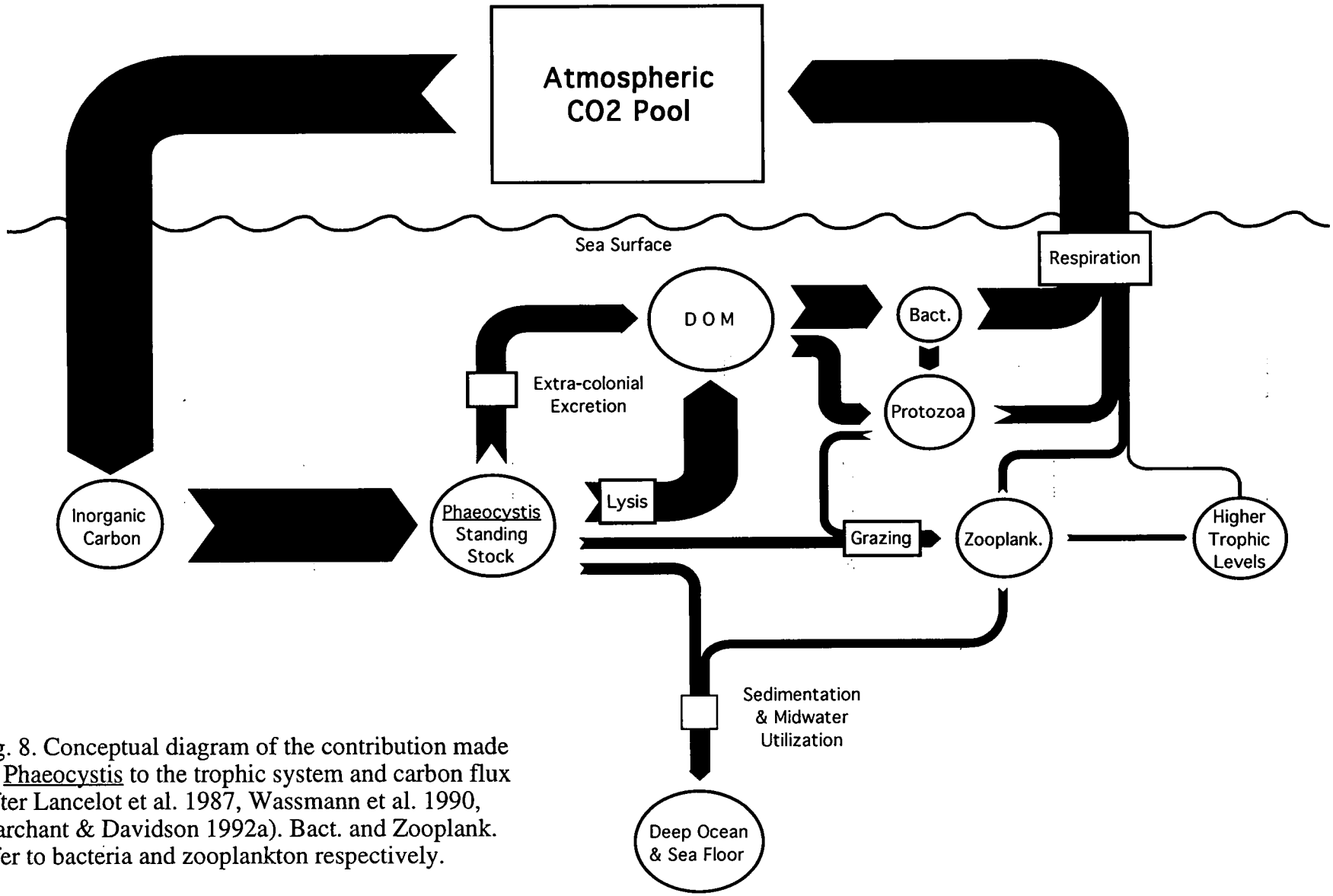


Fig. 8. Conceptual diagram of the contribution made by Phaeocystis to the trophic system and carbon flux (after Lancelot et al. 1987, Wassmann et al. 1990, Marchant & Davidson 1992a). Bact. and Zooplank. refer to bacteria and zooplankton respectively.

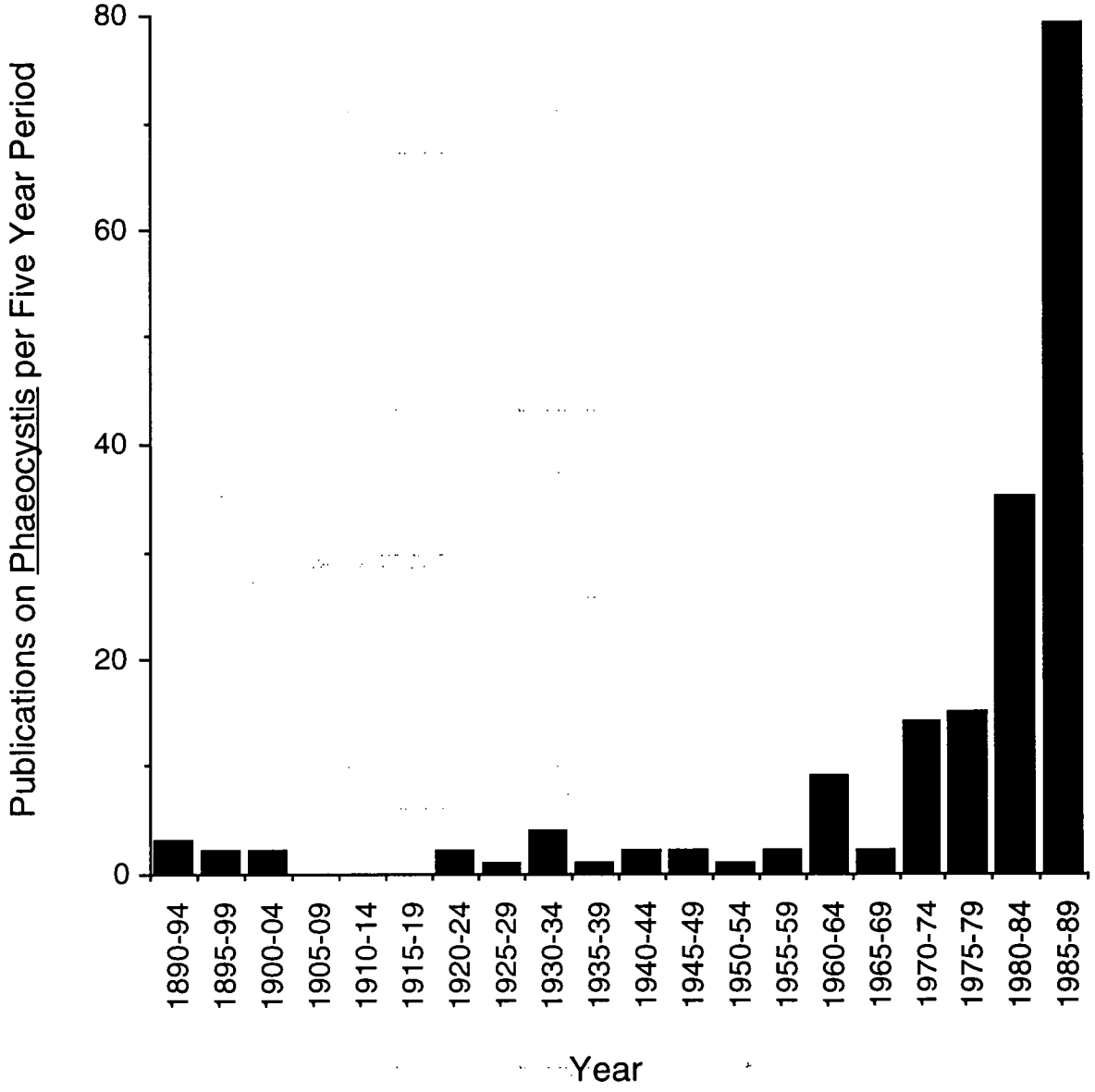


Fig. 9. Publications referring to Phaeocystis each five years period over the last 100 years showing the exponential increase in scientific attention devoted to this species.

It is, however, clear that Phaeocystis blooms have pervasive effects of on the biotic and abiotic marine environment. Chapter 4 demonstrates the effects of P. antarctica blooms in Antarctic waters and stresses the importance of any UVB–related change in its absolute or relative in abundance in Antarctic. Phaeocystis is often reported as dominating the phytoplankton (Chapter 3, section 4). However, the unique physiology of Phaeocystis means that blooms of this alga create an equally unique marine environment; one rich in particulate and dissolved organic matter but poorly linked to metazoan grazers (see section 5). Such a trophic function contrasts with the classical view of the Antarctic pelagic food web in which phytoplankton are grazed by krill, which are in turn consumed by such predators as whales, seals, penguins and sea birds (Marchant 1993). However, protozoa are able to utilise the abundant carbon produced by Phaeocystis (Chapter 3 section 5.4). The contemporary view of Antarctic marine microbial food web recognises that protozoan microheterotrophs such as choanoflagellates, dinoflagellates, ciliates, bodonids and euglenoids are abundant and important contributors pelagic trophic processes (eg. Garrison et al. 1983, 1991, Hewes et al. 1985, Marchant 1985, 1993, Lessard & Rivkin 1986, Bjørnsen & Kuparinen 1991, Garrison 1991a, Marchant & Murphy 1993). Chapter 4 examines the influence Phaeocystis antarctica exerts over the bacterial and protistan community structure, function and the carbon dynamics at an inshore site near Davis Station, Antarctica.

CHAPTER 4

Protist abundance and carbon concentration during a Phaeocystis–dominated bloom at an Antarctic coastal site

1. INTRODUCTION

Numerous studies have been conducted at Antarctic coastal sites and at ice edges to ascertain the species composition and abundance of protists (eg. Hoshiai 1977, Buck & Garrison 1983, Garrison et al. 1987, Lipski 1987, Perrin et al. 1987, Fryxell & Kendrick 1988, Garrison & Buck 1989a, Tanimura et al. 1990, Mathot et al. 1991, McMinn & Hodgson 1993, Kang & Fryxell 1993, Kivi & Kuosa 1994, Kang & Lee 1995). However, few reports have addressed the interactions between the various protists, bacteria and available carbon sources. Such studies suffer the inevitable limitation of not being able to follow a discrete, naturally occurring assemblage through time. However, though there need be no continuity between sample times, the protist species composition and chemical characteristics within each sample reflect the processes determining community structure and function. Comparisons with previous studies at this site (Perrin et al. 1987, Gibson et al. 1990, Marchant & Perrin 1990) indicate a high level of interannual consistency in bacterial abundance and the dominant phytoplankton and choanoflagellates. Thus, the processes determining community composition at this site repeatedly produce a similar sequence of species and abundance of organisms.

Primary production of the Southern Ocean supports substantial bacterial production. A clear relationship has been determined between bacterial and phytoplanktonic biomass at various localities around the Antarctic continent at different times of the year (eg. Fuhrman & Azam 1980, Hanson et al. 1983a, b, Kogure et al. 1986, Billen et al. 1987, Billen & Becquevort 1991, Thingstad & Martinussen 1991). However, little attention has been

given to changes in the abundance of Antarctic marine bacteria at a single location with time. Kottmeier and Sullivan (1990) suggest that bacterial growth in pack ice may mediate concentrations of nutrients and microheterotrophs and show that bacterial production is a significant source of carbon in the ice edge zone. Patterns of bacterial abundance in the water column vary but are reportedly dependant on substrate availability rather than limited by low ambient temperature (Billen & Becquevort 1991, Thingstad & Martinussen 1991). Satoh et al. (1989) found a single peak in bacterial numbers in December near Syowa station, while Gibson et al. (1990) described a double peak with maxima in November and February near Davis station with the concentration at the end of December being the lowest for any time of the year. Here I report the variation in bacteria, protists and carbon concentrations through the summer in Antarctic coastal waters near Davis and indicate the pivotal role of Phaeocystis antarctica in determining their abundance in this environment.

2. MATERIALS AND METHODS

Sampling was conducted in Prydz Bay at a site 5 km offshore from the Australian Antarctic station of Davis (68° 30' S, 77° 50' E). Temperature profiles from surface to the bottom depth of 100 m were obtained using a Yeo-Kal Model 606 Submersible Data Logger. 10 l water samples were obtained at a depth of 15 m by Niskin bottle at approximately weekly intervals between 15/11/88 and 21/2/89. All apparatus used for sampling water destined for chemical analysis was soaked in 5% Decon 90 detergent for no less than one week, thoroughly rinsed in MilliQ or Elgastat deionised water, then soaked in 5% HCl for the same time rinsing repeated. The contents of the Niskin bottle were thoroughly mixed before removing subsamples for counts of bacteria, phytoplankton and chemical analysis. All water samples were handled in a laminar flow hood.

Five hundred ml of sea-water was fixed with acid Lugol's solution and volumes between 15 ml and 500 ml were sedimented. Cells greater than 3 μm diameter were counted in fifteen replicate fields of view using an inverted microscope at 200 x magnification and the mean and standard deviation calculated. Eighty ml of water was fixed with 4%

glutaraldehyde and stored in sterile bottles. One to 20 ml subsamples, depending on bacterial concentrations, were stained with 4',6-diamidino-2-phenylindole (DAPI) (Porter & Feig 1980), filtered onto 0.1μm Nuclepore filters and total bacteria (epibacteria and bacterioplankton) counted by epifluorescent microscopy at 1000 x magnification.

Nonmetric multidimensional scaling (MDS) and cluster analysis were carried out on species abundance data (Field et al. 1982). Data for those autotrophs and heterotrophs occurring at more than 4% total abundance at any one sample time were used. As all data scales were identical, log data transformation ($Y_{ij}=\log(X_{ij}+1)$) was used when comparing samples and data were relativized ($Y_{ij}=100.X_{ij} / \Sigma_{j=1}^{n}.X_{ij}$) for the inverse comparison of species. The Bray-Curtis index of similarity was used and the data classified using group average sorting. The MDS starting configuration was generated by principal co-ordinates analysis and run over 75 iterations.

Two and a half litres of water, or at higher cell densities, until the filter clogged, were passed through 0.45 μm pore size acid-cleaned Millipore filters. Two 5 ml rinses of 3.6% w/v $(NH_4)_2CO_3$ and one of 1 mM Na_2 EDTA in 3.6% w/v $(NH_4)_2CO_3$ were then passed through the filter. The filter was then divided into eight, and two opposite segments analysed for carbohydrates (CHO) using the phenol sulphuric method (Marshall & Orr 1962). Another 500 ml of water was passed through 25 mm diameter Whatman GF/F filters that had been fired at 450°C for 16 hours. This filter was sectioned into eight and two of the segments analysed for carbon and nitrogen using a 185B Hewlett Packard Carbon Hydrogen Nitrogen analyser with a 3380A Hewlett Packard Integrator. A catalyst of 3 parts MnO_2: 1 part Cr_2O_3: 1 part diatomaceous earth was used to enhance oxidation at the furnace temperature of 800°C with a combustion time of 50 s. Approximately 20 ml of filtrate was stored at 4°C in glass vials with teflon lid inserts that had been cleaned with Decon 90 and acid prior to analysis for DOC.

DOC was measured using a Technicon Auto Analyzer II sampler, La Jolla PO-24 Photo-oxidation unit and Horiba PIR-2000 infrared gas analyser. Oxidation of DOC to CO_2 by

UV light was enhanced by saturation with ultra pure O_2. Ultra pure N_2 was used as the carrier gas to the infrared gas analyser.

3. RESULTS

3.1. Water Column Characteristics

Sea ice cover was 100% until 3/1/89 after which it declined to approximately 70% cover until 24/1/89 and approximately 50% thereafter (Fig. 1). Ice was completely absent by 1/2/89 but 70% cover had reformed by 21/2/89.

The temperature profile (Fig. 1) shows that the water column was unstructured until after 7/12/88. Between 22/12/88 and 17/1/89 surface temperature rose to its maximum of 1.13°C and the depth of warming increased with time. This thermal structuring of the water column occurred despite there being 70% - 100% ice cover and indicates the movement of water from nearby ice free areas to the sample site. Surface water temperature had begun to fall by the time the ice was absent from the sampling site.

3.2. Abundance of Organisms

Bacterial abundance was essentially bimodal with a smaller peak in November (Fig. 2A). The concentration increased until mid-December then declined rapidly until the end of the year whereupon it increased again to the highest values for the summer between 24/1/89 and 14/2/89 before again decreasing in late February.

Phaeocystis antarctica occurs both as motile, scale-covered flagellates and in gelatinous colonies. The colonial stage was observed in low numbers from the beginning of sampling and, although dominating phytoplankton numbers, remained in low abundance until mid December. Its abundance then increased dramatically to $6x10^7$ cells.l^{-1} on 3/1/89 before decreasing at about the same rate (Fig. 2B) but still remained numerically dominant until 8/2/89. Motile cells of P. antarctica increased in number at the same time as the colonial

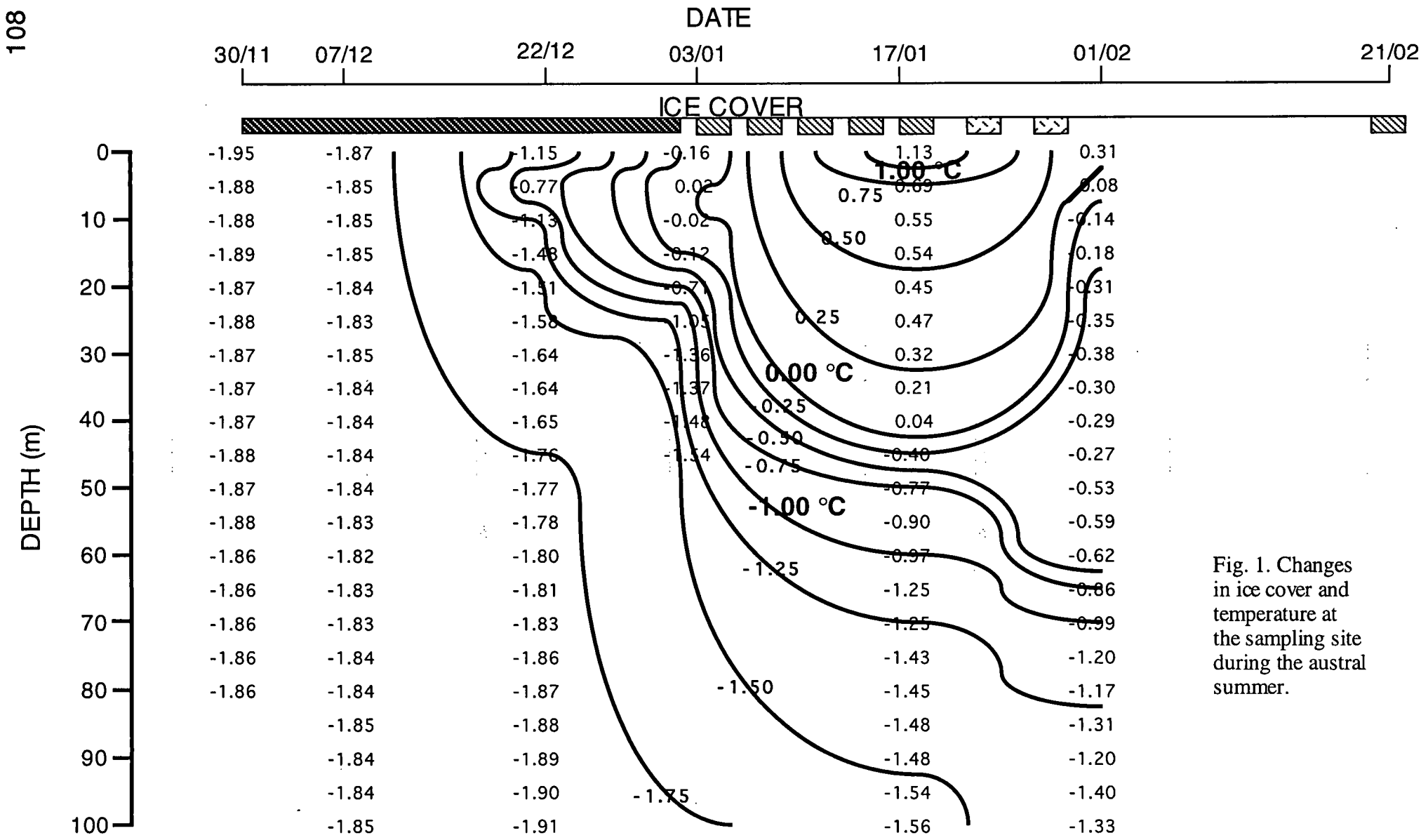


Fig. 1. Changes in ice cover and temperature at the sampling site during the austral summer.

form but declined until 10/1/89, during the bloom of the colonial stage, whereupon they increased to 3.4 x 10^5 cells.l^{-1} in late January (Fig. 2B).

The total diatom concentration began to increase at the same time as the colonial P. antarctica bloom, but their concentration did not increase between 27/12/88 and 3/1/89, the time when colonial P. antarctica was approaching and at maximum abundance. Diatom concentration continued to increase after the decline of the P. antarctica bloom, with a maximum of 5.9 x 10^6 cells.l^{-1} on 17/1/89 (Fig. 2C).

Taxonomic groups whose members consist partly or wholly of heterotrophs followed similar trends in abundance over the summer. Dinoflagellate concentration increased at the same time as colonial P. antarctica to approximately 7 x 10^4 cells.l^{-1} and persisted at around this concentration for the remainder of the summer with the exception of a further dramatic increase to a peak abundance of 5.4 x 10^5 cells.l^{-1} immediately after the P. antarctica bloom on 10/1/89 (Fig. 2D). Their numbers then declined to mid-Phaeocystis bloom concentrations by 17/1/89. Choanoflagellates bloomed between 27/12/88 and 24/1/89 reaching a maximum concentration of 1.2 x 10^6 cells.l^{-1} on 3/1/89. Their changes in abundance closely followed P. antarctica but persisted after its bloom (Fig. 2E). The principal ciliates were tintinnids and Strombidium Clarapede & Lachmann spp. (Fig 2F). The tintinnid population was dominated by Eutintinnus Kofoid & Campbell spp. while Codonellopsis Jörgensen sp. was occasionally observed. Eutintinnus was not seen before 3/1/89 when its concentration rose to 1.6 x 10^5 cells.l^{-1}. Its concentration then rapidly declined and beyond 10/1/89 remained less than 5 x 10^3 cells.l^{-1}.

Strombidium spp. (mainly S. sulcatus Claparede & Lachmann) were the only other ciliates observed and differed from other heterotrophs in not reach maximum concentrations during or immediately after the bloom of P. antarctica (Fig. 2F). Concentrations were never significantly above zero but low numbers were first observed on 3/12/90, increasing to approximately 5 x 10^3 cells.l^{-1} after the onset of the P. antarctica bloom and remained at

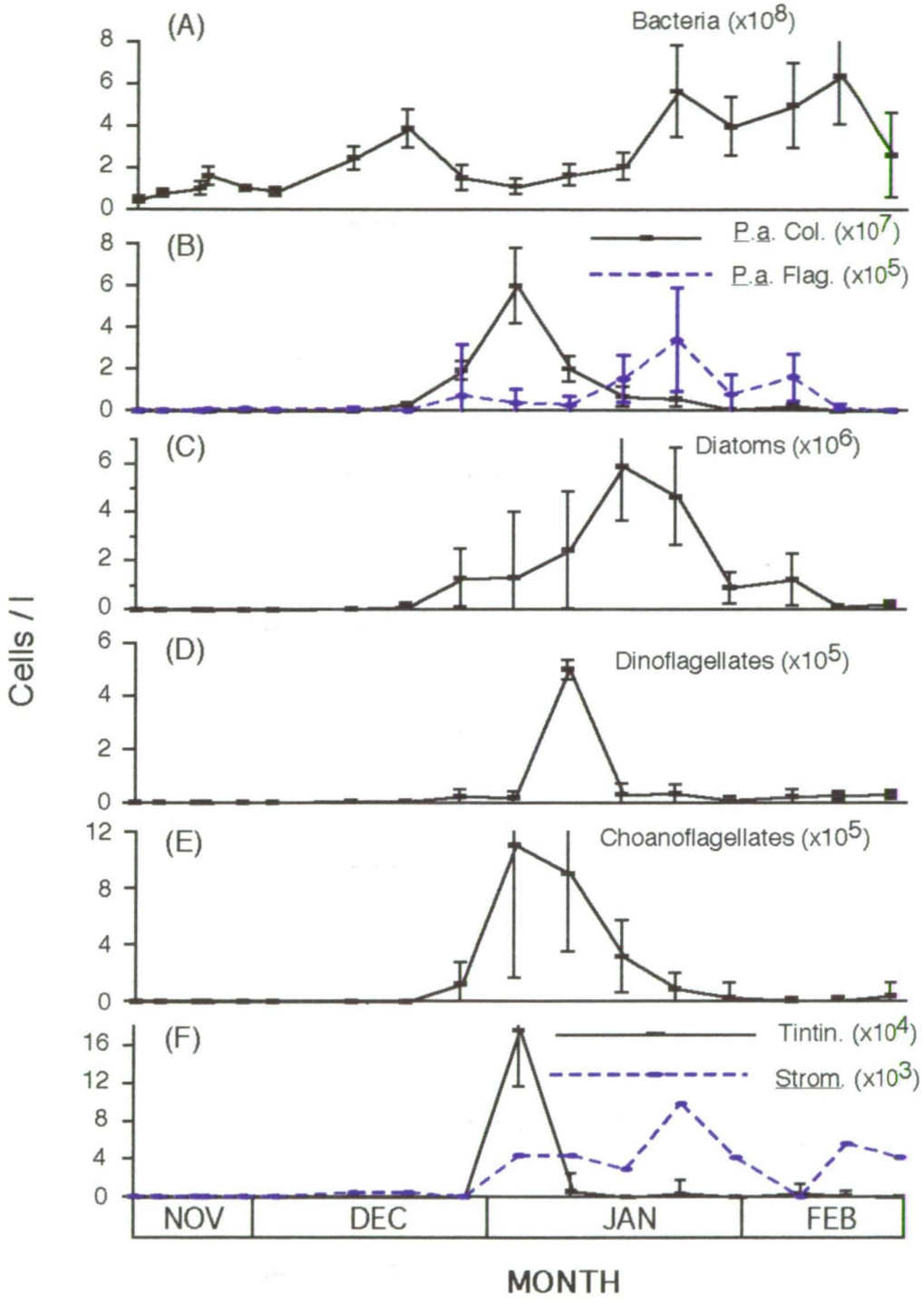


Fig. 2. Changes in the concentration of (A) bacteria, (B) Phaeocystis antarctica cells in the colonial (P.a. col.) and in the motile (P.a. flag.) phases of its life cycle, (C) total diatoms, (D) dinoflagellates, (E) total choanoflagellates, and (F) tintinnids (Tintin.)and Strombidium spp (Strom.) Error bars indicate ±1 standard deviation. Standard deviations for Strombidium were always greater than the mean and are not shown.

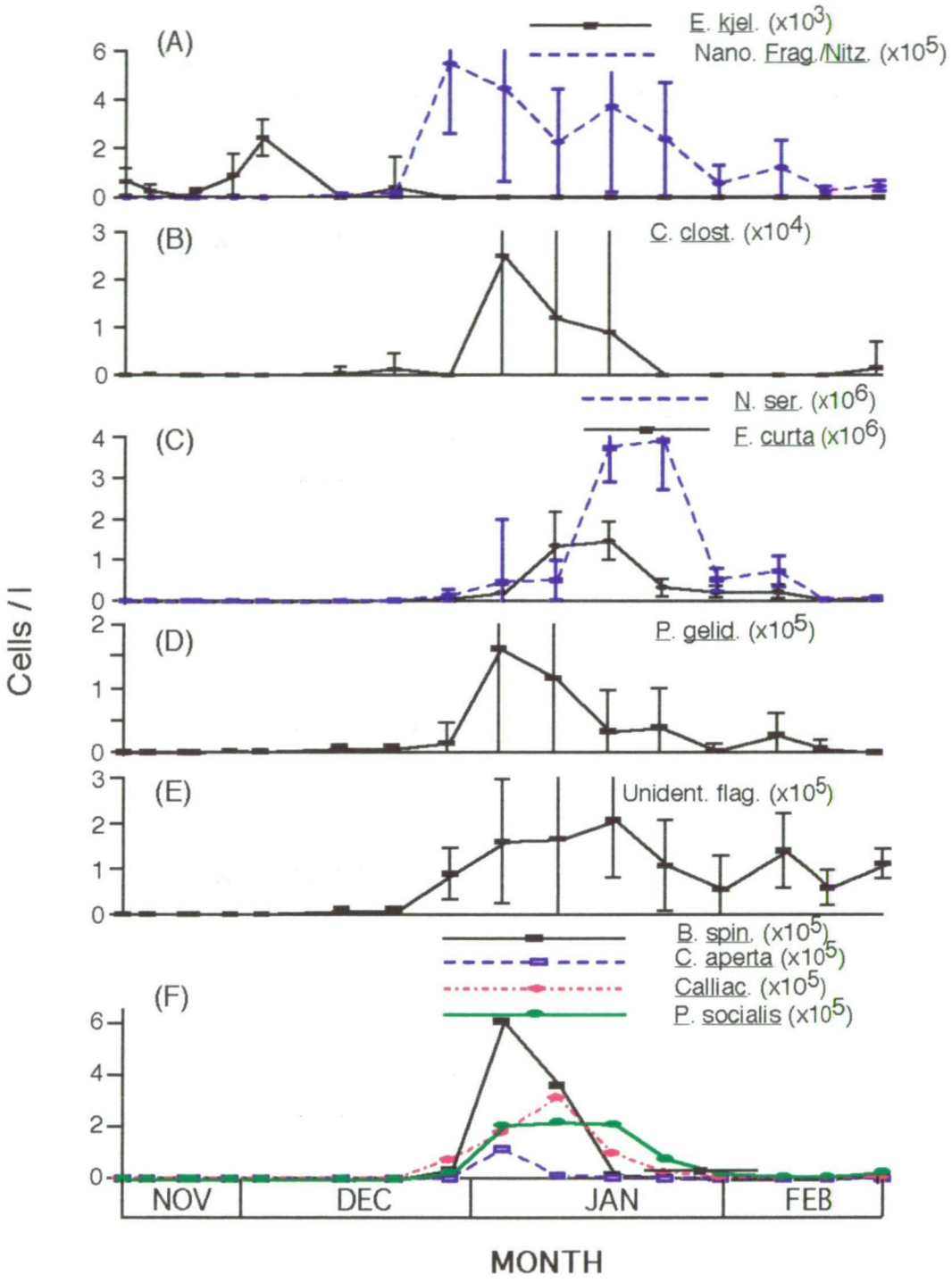


Fig. 3. Changes in the concentration of (A) Entomoneis kjellmannii (E. kjel.) and nanoplanktonic Fragilariopsis and Nitzschia spp (Nano. Frag./Nitz.), (B) Cylindrotheca closterium (C. clost.), (C) N. sp. c.f. seriata (N. ser.) and Fragilariopsis curta (F. curta), (D) Pyramimonas gelidicola (P. gelid.), (E) unidentified flagellates (Unident. flag.) and (F) choanoflagellate species Bicosta spinifera (B. spin.), Crinolina aperta (C. aperta), Calliacantha spp. (Calliac.), Parvicorbicula socialis (P. socialis). Error bars indicate ±1 standard deviation.

around this level for the remainder of the sampling time with a peak on 24/1/89 of 10^4 cells.l^{-1}.

Various species of diatoms proliferated at different times during the summer. Earliest was Entomoneis kjellmanii (Cleve) Poulin & Cardinal, a major component of the sea-ice community, which peaked on 3/12/88 but rapidly declined and was not seen for the rest of the summer (Fig. 3A). Nanoplanktonic Fragilariopsis and Nitzschia Hassall spp. concentration reached its maximum shortly before the bloom of colonial P. antarctica. Their concentration began to decline at the onset of the bloom of P. antarctica and continued to decline throughout the remainder of the summer (Fig. 3A). Cylindrotheca closterium Ehrenberg (Fig 3B) was exceptional among the diatoms in that it reached maximum concentration between 28/12/88 and 17/1/89, during the bloom of P. antarctica.

Nitzschia seriata Cleve is not recognised as an Antarctic species in the current taxonomic literature (Hasle & Medlin 1990). This was not the case at the time of this study. Thus, this species will be referred to as Nitzschia sp. c.f. seriata, and refers to the Nitzschia genus that form colonies, united into chains by overlapping cell apices. The concentration of morphologically similar chain-forming diatoms grouped within the species Nitzschia sp. c.f. seriata increased in concentration during the onset of the P. antarctica bloom and attained maximum abundance on 17/1/89 (Fig. 3C), immediately followed by Fragilariopsis curta (V. H.) Hasle. N. sp. c.f. seriata and F. curta were the major constituents of the diatom bloom. The minor constituents of the diatom assemblage, Chaetoceros dichaeta Ehrenberg, C. neglectum Karsten and C. simplex Ostenfeld, were most abundant during the P. antarctica bloom. Fragilariopsis cylindrus (Grunow) Hasle, Rhizosolenia Brightwell spp. and Nitzschia stellata Manguin contributed to the assemblage after the peak in P. antarctica concentration.

Peak abundance of the prasinophyte Pyramimonas gelidicola McFadden, Moestrup & Wetherbee (1.6×10^5 cells.l^{-1}) coincided with the bloom of the colonial stage of P. antarctica and persisted longer (Fig. 3D). Auto- and heterotrophic flagellates, of which

most were nanoplanktonic and the most numerous was Micromonas Manton & Parke sp., increased in concentration at the same time as P. antarctica , reaching a peak concentration of 2 x 10^5 cells.l^{-1} on 17/1/89 (Fig. 3E). The choanoflagellate population was dominated by Bicosta spinifera (Thronsden) Leadbeater, Crinolina aperta (Leadbeater) Thomson, Calliacantha Leadbeater spp. and Parvicorbicula socialis Thomson, the latter commonly in aggregates (Fig. 3F). The concentration of B. spinifera increased during the onset of the Phaeocystis bloom and B. spinifera and the previously unobserved P. socialis attained maximum concentrations during the peak in P. antarctica on the 3/1/89, whereupon they declined rapidly. C. aperta and Calliacantha spp. persisted longer than B. spinifera and P. socialis and peaked in abundance upon the decline of P. antarctica on the 10/1/89.

3.3. Assemblages of Organisms

Figure 4A and B show the samples which were grouped together from their species abundance using cluster analysis and MDS respectively. Separation of sample dates at a similarity of 0.87 in cluster analysis gave identical groupings to that obtained by MDS. MDS results are adequately depicted in 2 dimensions (2-D) as stress only rose from 5.18 to 8.33 when passing from 3-D to 2-D plotting. Group 1 contained samples between 15/11/88 and 5/12/88 where auto- and microheterotroph concentrations were low and bacterial concentrations moderate. Group 2 comprised two samples, 13/12/88 and 20/12/88, when phytoplankton concentrations were increasing towards the bloom of colonial P. antarctica but microheterotroph concentration was low and bacterial concentrations increased. Group 3 contained samples from 27/12/88 to 14/2/89 and encompassed the bloom of phytoplankton and heterotrophs. The sole sample in group 4 was taken on the 21/2/89, the last sample of the summer when P. antarctica was absent for the first time. Pyramimonas gelidicola and tintinnids were also absent and bacterial concentrations declined from their peak.

Inverse analysis gave species groups using cluster analysis (Fig 4C) and MDS (Fig 4D). Delineation of species groups at a similarity index of 0.69 gave six groups again identical

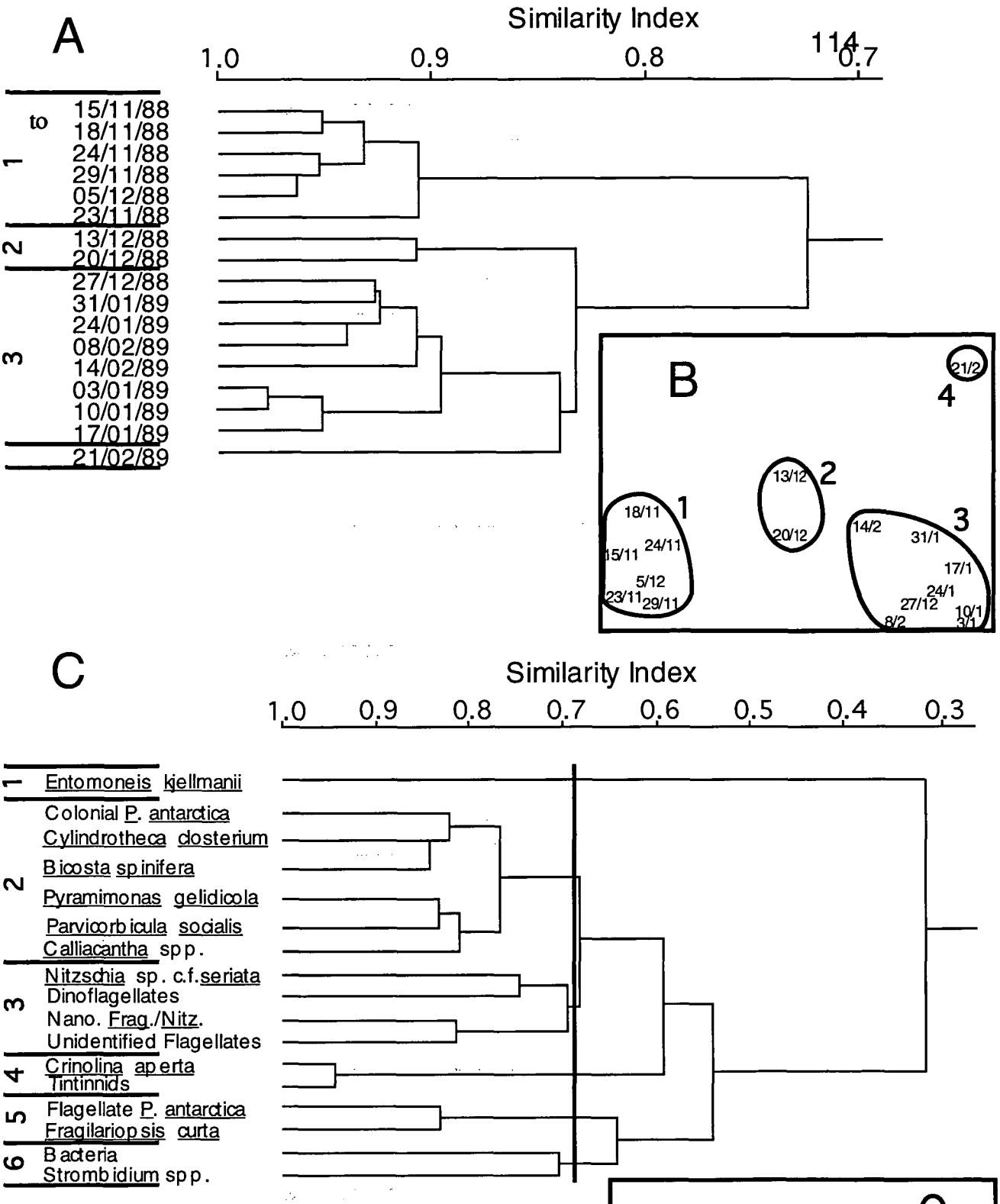


Fig. 4. Sample grouping by (A) cluster analysis and (B) MDS using log transformed data, the Bray-Curtis index and group average sorting. Species grouping by (C) cluster and (D) MDS as above but using relativized data. MDS species are 1. Phaeocystis antarctica colonial, 2. P. antarctica flagellate, 3. Entomoneis kjellmanii, 4. Cylindrotheca closterium, 5. Nitzschia sp. c.f. seriata, 6. Fragilariopsis curta, 7. Nanoplanktonic Fragilariopsis/Nitzschia spp., 8. Pyramimonas gelidicola, 9. Unidentified flagellates, 10. Bacteria, 11. Strombidium spp., 12. Bicosta spinifera, 13. Crinolina aperta, 14. Calliacantha spp., 15. Parvicorbicula socialis, 16. Dinoflagellates, 17. Tintinnids.

to those obtained by MDS. 2-D was an adequate depiction of MDS as stress only rose from 5.23 to 8.13 on going from 3-D to 2-D. Group 1 consisted of E. kjellmanii alone. This was the only species to attain maximum numbers in group 1 of the sample comparisons. Group 2 contained those species that had a relatively brief peak in abundance about the time of the peak of colonial P. antarctica and which were present in most samples but at much lower concentrations than their peak abundance. These species were colonial P. antarctica, Cylindrotheca closterium, Pyramimonas gelidicola, Bicosta spinifera, Parvicorbicula socialis and Calliacantha spp. Those partly or wholly autotrophic taxa of which the populations declined or increased only slowly during the P. antarctica maximum comprised group 3 namely, Nitzschia sp. c.f. seriata, dinoflagellates, nanoplanktonic Nitzschia spp. and unidentified flagellates. Crinolina aperta and tintinnids were very closely related to form group 4. Both species were essentially absent until their peak on the 3/1/89 followed by a rapid decline. Group 5, containing flagellate P. antarctica and Fragilariopsis curta, showed the same decline in growth rates as group 3 but their peak in abundance occurred on 24/1/89, three weeks after the peak in colonial P. antarctica. Species group 6, which was composed of Strombidium and bacteria, persisted from early season until the end of sampling with peak abundance in January and February.

3.4. Organic Carbon and Nitrogen

Three main peaks of DOC occurred during the summer (Fig. 5A). The first on 24/11/88 occurred before the appearance of any major primary producers in the summer and is likely to have been released from the sea-ice. At the time of this DOC peak is also a peak of POC (Fig. 5B) and PCHO (Fig. 5C). The major peak of DOC, which reached a maximum in excess of 100 $mg.l^{-1}$, POC and PCHO coincided with the bloom of colonial P. antarctica and persisted from around 20/12/88 to 31/1/89. The third peak of DOC (80 $mg.l^{-1}$) occurred on 14/2/89, a time of declining POC and PCHO. The concentration of POC and PON declined steadily from the major peak of 760 $\mu g.l^{-1}$ and 107 $\mu g.l^{-1}$ respectively on 10/1/89 (Fig. 5B). Before 20/12/88 concentrations of POC and PON were too low to

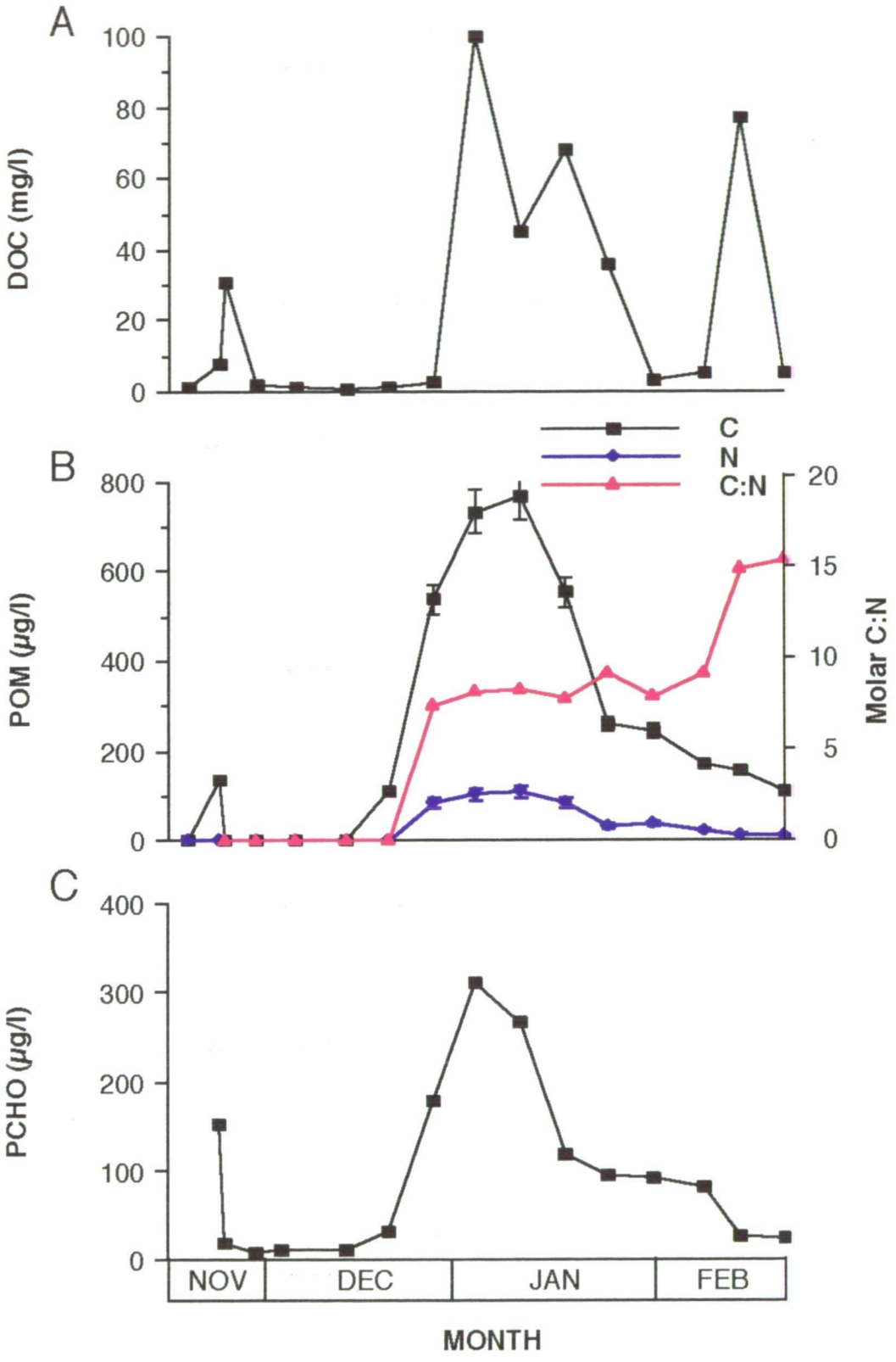


Fig. 5. Changes in the concentration of (A) dissolved organic carbon (DOC), (B) particulate organic matter (POM) showing carbon (**C**), nitrogen (**N**) and the molar ratio of carbon to nitrogen (**C:N**) and (C) the concentration of glucose equivalent particulate carbohydrate (PCHO). The absolute concentration of DOC on 3/1/89 is unknown but was greater than 100 mg.l-1.

calculate the C:N ratio. After this the molar C:N ratio was approximately 7:1, approximately that of the Redfield ratio, and remained at this value until 8/2/89 after which it increased to around 13:1 (Fig. 5B).

4. DISCUSSION

The timing of peaks in abundance of bacteria, autotrophic and heterotrophic protists and changes in the concentration of DOC, POC and PON appear closely related during summer at this coastal Antarctic site. The interannual recurrence of similar species and abundance (Perrin et al. 1987, Gibson et al. 1990, Marchant & Perrin 1990) suggest that the biotic and abiotic events determining the community at this site are not spatially or temporally isolated. It also demonstrates that this study, based on a single site and depth, is representative of protistan community dynamics in this area. The concentrations changed rapidly on occasions during this study. This clearly does not infer rates of production from repeated sampling of a discrete population but instead indicate associations of organisms in water masses as they pass the fixed sampling site. This does not preclude discussion of successional sequences as planktonic succession is, by necessity, defined in these terms. Nor does it preclude discussion of the interactions between species as it is those interactions that determine the composition of the community within each sample. Such single point sampling has been used to ascertain successional sequence in other sites from which species interactions have been inferred (eg. Jeffrey 1981).

4.1. Bacterioplankton

The November peaks of bacteria, Entomoneis kjellmanii DOC, POC and PCHO, when the sea was entirely ice covered and the water column was thermally unstructured, was probably due to release of organic substrates from the sea-ice (Garrison & Buck 1989b). Phaeocystis secrets a large proportion of its photoassimilated carbon (Guillard & Hellebust 1971) and these extracellular products are a suitable substrate for bacteria (Eberlein et. al. 1985, Veldhuis et. al. 1986b, Davidson & Marchant 1987, Verity et al. 1988b). However,

the bloom of this alga after 20/12/88 coincides with a decline in bacterial concentration confirming observations by Laanbroek et al. (1985) and Lancelot and Billen (1984) that bacterial concentrations are depressed by actively growing Phaeocystis. However Gibson et. al. (1990) found that the December minimum in bacterial numbers were not concurrent with the peak of this alga and was attributed to grazing alone. Bacterial abundance is reportedly related to substrate availability and is relatively unaffected by the low ambient temperature in Antarctic waters (Billen & Becquevort 1991, Thingstad & Martinussen 1991). Thus, the decline in bacterial abundance between 20/12/88 and 17/1/89, likely to result initially from the release of a bacteriocide by actively growing P. antarctica (Sieburth 1960, Davidson & Marchant 1987) followed by microflagellate grazing, occurred at the time of PCHO abundance and the seasonal maximum of DOC. The abundance of DOC (in excess of 100 $mg.l^{-1}$) during the P. antarctica bloom demonstrates the incapacity of bacteria to fully utilize this substrate.

The January increase in bacterial concentration accelerated greatly after the microheterotroph bloom. This agrees with Fenchel (1982), Garrison and Buck (1989a) and Sherr et al. (1989) who have shown microheterotroph grazing is a principal determinant of bacterial mortality. The coincidence of the peaks of DOC and bacteria in early February indicates that a direct relationship between the two is sustainable when microheterotroph grazing is minimal.

The delay between the substrate production by phytoplankton and the bacterioplankton peak at a single sample site was found to be about 10 days in the North Sea (Billen & Fontigny 1987), 11 to 18 days off Newfoundland (Pomeroy & Deibel 1986) and about one month in Antarctic waters (Billen et al. 1987). The discrepancy in correlation of phyto- and bacterioplankton biomass showed that, in comparison with temperate waters, bacterial response to substrate availability in polar and subpolar waters was delayed. My data also showed a one month delay between the peak concentration of phytoplankton and bacteria at the sample site but clearly indicate that the relationship is not simple.

4.2. Protists

Protist abundance attained its maximum as sea-ice cover declined. Major release of organic carbon and inoculation of the water column with protists from the ice biota has been observed (Garrison & Buck 1989b, Garrison & Gowing 1992). Such a release could have contributed significantly to the sudden increase in concentration of Phaeocystis antarctica, organic carbon and protozoa that was observed during the ice breakup in early January. However, Riebesell et al (1991) and Mathot et al. (1991) found negligible seeding of the water column with phytoplankton as a result of ice melt and observed that most ice algae sedimented or were grazed. Thus, high concentrations of P. antarctica and organic carbon may be ex situ production transported to the sample site from the east by coastal currents. The high concentration of flagellate P. antarctica early in the season followed by a decline during the bloom of the colonial stage support observations by Kornmann (1955) and Kayser (1970) that they may be a source of colonies. In addition, as reported by Verity et al. (1988b), the flagellate stage was observed being liberated from colonies at the end of the colonial bloom. This life-cycle change led to the increase in flagellate concentration in late January.

The peculiar physiology of Phaeocystis is regarded as strongly influencing the structure and function of ecosystems in which it predominates (Lancelot et al. 1987). The data presented in this chapter support the observation of previous authors (eg. Jones & Haq 1963, Smayda 1973, Chang 1983, Barnard et al. 1984, Admiraal & Venekamp 1986, Bätje & Michaelis 1986, Veldhuis et al. 1986b, Weisse et al. 1986) that Phaeocystis has an antagonistic effect on other phytoplankton species. This effect was sufficiently pronounced to cause those autotrophs whose numbers remained constant or declined during the P. antarctica bloom to be separated by cluster analysis and MDS. Production of UV-B absorbing pigments by colonial P. antarctica (Marchant et al. 1991) may have profound effects on the timing of the abundance in these waters. In addition, manganese and iron concentrations in Southern Ocean are very low (Martin et al. 1990) and the high manganese

accumulation reported for colonial Phaeocystis (Morris 1971, Davidson & Marchant 1987, Lubbers et al. 1990) may mediate the succession of Antarctic marine autotrophs.

At the time of greatest protist abundance (cluster and MDS sample group 3) species were divided into groups 4, 2 and 3 representing those species that peaked during the P. antarctica maximum only, high P. antarctica and post–P. antarctica bloom environment respectively. While the abundance of most autotrophic populations did not increase during the bloom of P. antarctica, protozoa proliferated. The coincidence of the Phaeocystis and tintinnids peaks has also been observed by Admiraal and Venekamp (1986) who reported blooms of tintinnids immediately following Phaeocystis blooms, demonstrated that they were grazing Phaeocystis and concluded that microfaunal grazing may limit the duration of the Phaeocystis bloom. Choanoflagellates attained concentrations approximately an order of magnitude higher than those reported for the Weddell Sea by Buck and Garrison (1988). Dinoflagellates, of which a large proportion may be heterotrophic (Garrison & Buck 1989a), also peaked at this time.

After the peak of P. antarctica, microheterotroph concentrations declined sharply. This was not due to insufficient carbon as the DOC and POC concentrations in these samples was only slightly lower than those in early January. Direct uptake of primary production by microheterotrophs has been reported (Fenchel 1987) including utilization of DOC sources (Sherr 1988, Marchant 1990, Marchant & Scott 1993). The microheterotroph maximum at the time of highest organic substrate availability and low bacterial abundance strongly suggests direct uptake of these substrates. Hewes et al. (1985) suggested that microzooplankton form a important link to higher trophic levels in Antarctic waters and what Lampert (1978) described as "sloppy feeding" by zooplankton may have contributed to the increase in DOC at the time of low microheterotroph abundance. P. antarctica, by simultaneously producing copious organic carbon and inhibiting of bacterial growth, appears to increase the emphasis on microheterotrophs as the link to higher trophic levels.

The ciliate Strombidium is the exception to the pattern observed in the other heterotrophic groups. MDS and cluster analysis demonstrate that this organism is only poorly linked with the abundance of other protists. This probably reflects the range of particle size available to it, making it less dependent on the trophic strictures applied to the other heterotrophs, together with its capacity to function as a facultative mixotroph (Stoecker et al. 1987).

4.3. Organic Compounds

Discrimination of POC and DOC produced by Phaeocystis is equivocal. Most of the biovolume of its blooms is due to the colony matrix of which significant proportions can be lost by filtration (Verity & Smayda 1989, Veldhuis & Admiraal 1985, Lancelot 1984b, Bölter & Dawson 1982). Filtration to dryness and rinsing with ammonium carbonate employed in this study would probably have lost much of the mucilage in the particulate fraction, hence significantly underestimating the POC. This is borne out by the relatively low PCHO concentration given that the DOC indicates very high carbon exudation rates. Though filtration pressures were low (below 100 mm Hg) mucilage is likely to have been lost to the DOC fraction. However, this effect was minimised by obtaining the DOC sample before the filter dried. The extremely rapid changes in organic carbon and nitrogen (requiring production rates around 20 g $C.m^{-3}.day^{-1}$) are unrealistically high, demonstrating that this production did not occur in situ.

Measured POC and PON concentrations obtained during this study are similar to those obtained during a bloom of 2.8×10^7 $cells.l^{-1}$ along the North Wales coast (Claustre et al. 1990). DOC concentrations of up to 20 $mg.l^{-1}$ were measured during a P. antarctica bloom by Bölter and Dawson (1982) and cell concentrations in this study are greater than any previously reported. POC concentrations were over two orders of magnitude less than the DOC at the peak of the P. antarctica bloom and this persisted, though to a lesser extent, for the remainder of the period when P. antarctica dominated. It is evident from the accumulated POC, PCHO and DOC concentrations were observed that organic substrates,

particularly DOC, are available far in excess of its utilization. Claustre et al. (1990) also found that the greatest part of the biomass of Phaeocystis blooms was lost from the food web linking phytoplankton to copepods. Wassmann et al. (1990) concluded that grazing on Phaeocystis was insufficient to control the magnitude of its bloom. In Antarctica Bölter and Dawson (1982) also found that heterotrophy was insufficient to utilize the DOC pool during the periods of intense primary production of a P. antarctica bloom. This study showed that more than 99% of the organic carbon existed as DOC and that this was largely unutilized at the sample site. Thus, previously published models of carbon flux which include Phaeocystis blooms (Billen & Fontigny 1987, Billen et. al. 1987) and those describing flux within individual colonies (Lancelot & Mathot 1985) differ greatly from this study.

It has been suggested that P. antarctica blooms result in the limitation of other autotrophs by depletion of trace metals (Davidson & Marchant 1987). Using the PON concentration and extrapolating from that of DOC to dissolved organic nitrogen, this study also indicates that nitrogen limitation would be expected during the bloom. Major depletion of nitrate was observed at the sample site about the time of the P. antarctica peak (A. McTaggart pers. comm.). Thus, though Antarctic phytoplankton are classically regarded as not being macronutrient limited, like McMinn and Hodgson (1993) in coastal fjords and Nelson and Treguer (1992) at the ice edge, brief periods of very high cell concentrations such as those observed during this study may well result in macronutrient limitation of phytoplankton growth.

5. CONCLUSION

Phaeocystis antarctica apparently influences the abundance of autotrophic species. The massive concentrations of carbon present during a P. antarctica bloom are apparently not significantly exploited by bacteria as their numbers are low at this time. The bloom of choanoflagellates, dinoflagellates and tintinnids during and immediately following the P. antarctica maximum suggests that a large proportion of the carbon passing to higher trophic

levels is channelled through the microheterotrophs. Heterotrophy is advantageous to Antarctic organisms surviving some nine months of the year in a substantial absence of light. When light and carbon are at their maximum these organisms are apparently able to utilize this resource, largely unconstrained by bacterial activity. As a dominant member of the phytoplankton, a major source of carbon and a bacteriocidal source P. antarctica is also a crucial determinant of the heterotrophic community. This study emphasises the importance of this species in Antarctic coastal waters.

The effect of UVB radiation on phytoplankton has been reviewed (Chapter 1). So to has the biology and ecology of phytoplankton in Antarctic waters (Chapter 2), with special reference to the exceptional role of Phaeocystis (Chapters 3 & 4). Thus, the foundation upon which the following experimental studies of the UVB photobiology of Phaeocystis and selected Antarctic marine diatoms has been established. Subsequent chapters have been published since 1991 and reflect the status of UV–related research at the time. The thesis text has been updated to include pertinent research since publication.

Like many phycologists, the physiological peculiarities of Phaeocystis have fascinated me for many years. We have seen that P. antarctica is frequently the first species to bloom in Antarctic waters were it is often reported as forming dense blooms in shallow waters (eg. Buck & Garrison 1983, Palmissano & Sullivan 1985, Fryxell & Kendrick 1988, Davidson & Marchant 1992b). This apparent vulnerability to UV exposure should have aroused suspicions that this alga possessed effective UV tolerance mechanisms. However, a casual inquiry by Dr. Graham Kelly led to the first discovery of high concentrations of UV–absorbing compounds in P. antarctica and this thesis being undertaken. Chapter 5 reports the discovery of UV–absorbing compounds and relates their concentration to life stage, geographic location and survival when exposed to UV radiation.

CHAPTER 5

UVB protecting pigments in the marine alga Phaeocystis antarctica

1. INTRODUCTION

Ozone depletion over Antarctica has occurred between September and November since the mid-1970s (Stolarski et al. 1986) and now persists until February (Jones & Shanklin 1995). This depletion has increased UVB irradiances (280 - 320 nm) reaching the Earth's surface during spring and summer (see Chapter 1, section 3). Solar UVB radiation penetrates seawater to depths that are able to influence the growth of macrophytes and phytoplankton (Jerlov 1950, Calkins & Thordardottir 1980, Worrest 1983, Maske 1984, Wood 1989); reaching depths in excess of 50 m in Antarctic waters (Gieskes & Kraay 1990, Karentz & Lutze 1990, Smith et al. 1992).

Phytoplankton production in the Southern Ocean occurs in environments vulnerable to UVB radiation at a time when irradiances at these wavelengths are enhanced by stratospheric ozone depletion. Sea-ice around Antarctica commences the southward retreat from its maximum extent in late September (Jacka 1983). Increasing insolation at this time leads to proliferation of algae both within the sea-ice and the marginal ice-edge zone where the development of a shallow pycnocline limits vertical mixing and promotes the development of blooms high in the water column (Smith 1987). Sea-ice algae contribute 10 - 50% of the primary production in some areas (Voytek 1989) but Antarctic sea ice in spring can be sufficiently transparent to UV that biologically significant doses are received by the ice algal community (Trodahl & Buckley 1989, Ryan 1992, Ryan & Beaglehole 1994). Melting sea-ice forms a shallow pycnocline in the marginal ice zone (MIZ) which may confine phytoplankton to depths of 20 m or less for up to 6 days (Mitchell & Holm-Hansen 1991, Veth 1991). Phytoplankton inhabiting the shallow

mixed depths of the marginal ice zone (MIZ) support 25 - 67% of the phytoplanktonic production in the Southern Ocean (Smith & Nelson 1986).

Survival, growth and photosynthesis of phytoplankton in the MIZ are reduced by UVB exposure (see Chapter 1, section 6.1.1 & 6.1.2) as they coincide with the springtime ozone depletion (Helbling et al. 1994). However the impact of UV radiation on Antarctic marine phytoplankton is equivocal (Roberts 1989, Voytek 1990). Opinions regarding the magnitude of the effect range from insignificant (Holm-Hansen et al. 1989b) to catastrophic (El-Sayed et al. 1990). The impact of ozone depletion on phytoplankton will depend on the magnitude and duration of exposure to UVB, their present tolerance and their ability to adapt to higher levels of UVB (Bidigare 1989, Karentz 1991).

Many organisms that inhabit environments subjected to high incident UV radiation produce UV–absorbing pigments to shield them from exposure to these wavelengths (see Chapter 1, section 5.2). The nanoplanktonic haptophyte Phaeocystis antarctica is abundant in Antarctic waters (Fryxell & Kendrick 1988, Vincent 1988, Davidson & Marchant 1992a, Marchant 1993) and is one of the first organisms to bloom in the ice and in the upper 10 m of the water column following the breakup of the sea-ice (Garrison et al. 1987, Fryxell & Kendrick 1988) and is arguably the most abundant and widespread phytoplankter of the Antarctic marine ecosystem . This chapter reports the finding of UV–absorbing pigments in Phaeocystis antarctica and demonstrate that they mitigate UV damage to this alga.

2. MATERIALS AND METHODS

A predominantly flagellate (strain DE10) and two colonial axenic strains (A1-3 & A1-4) of Phaeocystis antarctica, isolated from Prydz Bay, Antarctica (Antarctic Division Culture Collection, Hobart, Tasmania) and a unialgal strain (165-7) isolated from the East Australian Current (CSIRO Culture Collection of Micro-algae, Hobart, Tasmania) were maintained in GP5 medium (Loeblich & Smith 1968) in glass flasks at 4 and 20°C respectively on a 12 h light : 12 h dark cycle under cool white fluorescent tubes at an

intensity of 6.19±0.76 W.m^2 with no UVB enhancement. An aliquot of 50 ml was removed from each strain at mid–exponentially growing phase and was concentrated to 5 ml by centrifugation at 200 x g for 50 min at their incubation temperature. The predominantly flagellate strain was first filtered through 20 µm mesh netting to remove any colonies before the cells were similarly concentrated by centrifugation. Absorbance was measured by monochromatic scanning using an Hitachi 3200 spectrophotometer.

The proportion of viable Phaeocystis antarctica cells in Axenic Colonial A1-3, Axenic Flagellate DE10 and Colonial 165-7 strains was examined following exposure to enhanced levels of UV radiation. Cultures in exponential phase were illuminated with either increasing total irradiance or increasing UVB only (omitting Strain DE10). Cool-white fluorescent tubes and FS20T12-UVB Westinghouse Sunlamps (Fig. 1A) gave photosynthetically active radiation (PAR) of 3.99±1.00 x 10 W.m^2, UVA at 0.70±0.36 W.m^2 while the intensity of UVB was varied by attenuation with glass screens and the polystyrene culture flask (Lux) in which the organisms were grown (Fig. 1B). Spectral emission of UVB the Sunlamps (Fig. 1A) was measured at 1 nm intervals from 250 to 400 nm using a Macam spectroradiometer. An Haraeus CPS Suntest Xenon arc UV/white light source which simulates the spectral distribution of solar radiation was used to generate increasing total irradiance (Fig. 1A). The spectral emission of the Haraeus lamp is obtained from instrument specifications as the emission at the time experiments were conducted is unknown. The irradiance to which the cultures were exposed was measured with an International Light IL 1700 Radiometer equipped with detectors to measure PAR, UVA and UVB (Fig. 1C). The IL 1700 measures broad-band irradiance and erythemally weighted UVB. Though relatively inexpensive, rugged and easily operated in the laboratory and field it does not give accurate spectral data for calculation of UV dose. Primary calibration of detector response was made using a National Institute of Standards and Technology intercomparison package (NIST Test # 534/240436-88) with further calibration using four International Light primary transfer standards. Control cultures were unirradiated with UV. After incubation for 48 h under the 12 h light : 12 h dark regime (ie. 6, 12 and 6 h light

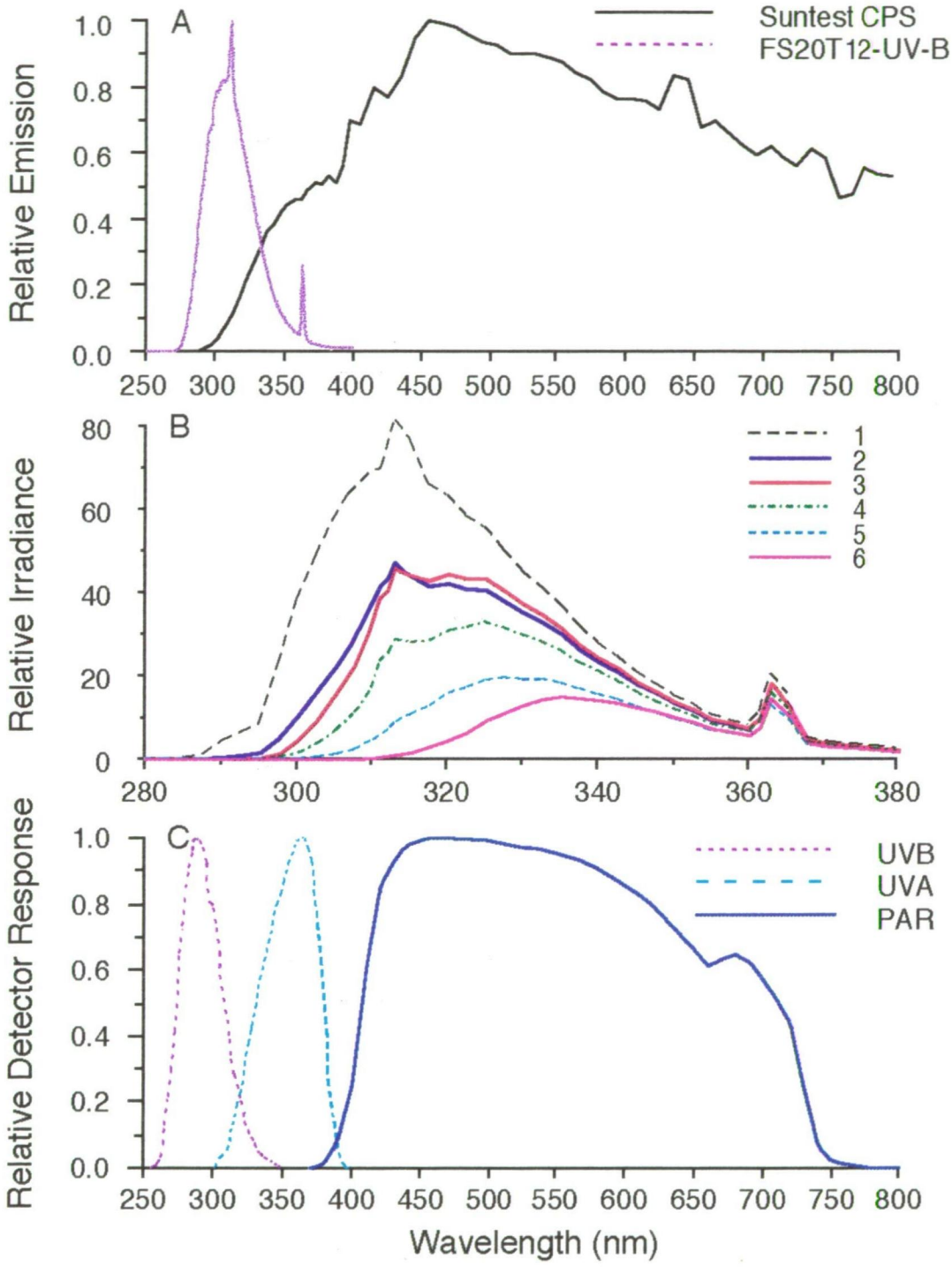


Fig. 1. Emission spectra of (A) Haraeus Suntest UV/white light source and FS20T12-UV-B Westinghouse sunlamps (redrawn from instrument specifications) and (B) Spectral distribution experienced by algae in experiments with increasing UV-B only. Relative irradiance was calculated from relative emission (maximum emission = 1) of UV-B fluorescent tubes (Westinghouse instrument specifications) x spectrophotometric transmittance of attenuating screens used in the experiments including attenuation due to polystyrene culture flask. Curve 1 polystyrene flask alone, curves 2 - 6 increasing attenuation by glass screens. All experimental UV-B irradiances were obtained using a single UV-B fluorescent tube except the two highest irradiances (not plotted) which had spectral distributions 1 and 2 but required two UV-B tubes. (C) The wavelength response of detectors used to measure PAR, UV-A and UV-B (redrawn from instrument specifications).

exposures with intervening 12 h dark periods) aliquots of culture were fixed with Lugols iodine, sedimented and the percentage of living cells and standard error determined using a minimum of 300 cells over 5 replicate fields of each treatment.

The long-term viability of irradiated cells was tested using four exponential-growth-phase cultures of Phaeocystis antarctica (Strain A1-3), which were exposed to irradiances spanning the UVB irradiances range under the same irradiation regime as used above plus an unirradiated control. A 30 ml aliquot from each irradiance treatment was added to 70 ml of fresh medium and the number of live cells in 15 replicate fields counted on days 0 and 13. Growth rates of all treatments were calculated (Verity et al. 1988a) and that of the unirradiated control used to predict the initial concentration of viable cells in irradiated treatments from the observed final cell concentration.

Quantitative measurements of UVB absorption were made using samples concentrated by centrifugation and extracted for 30 min at 50°C in growth medium (Scherer et al. 1988). It was established that 30 min was the extraction time at which there was maximum recovery of UVB–absorbing compounds. Peak height from these extractions was obtained by measuring the absorbance at 271 and 323 nm and subtracting the absorbance at these wavelengths from a line tangential to the absorbance minima around 250 and 380 nm. This removed the non-labile background absorbance observed in Fig. 2B.

Exponentially growing colonial Phaeocystis antarctica (A1-3), and Phaeocystis from Tasmanian coastal waters (PE2, CSIRO Culture Collection of Microalgae, Hobart, Tasmania), East Australian current (165-7) and the North Sea and English Channel (Strains Veldhuis and 540, Plymouth Culture Collection, England) were grown as for aqueous concentrates (ie with no UV radiation), and were extracted at 50 °C for 30 min in the culture medium; the 323 nm absorbance was calculated per unit chlorophyll a concentration of the culture. Chlorophyll a was extracted with methanol (Wright & Shearer 1984) and its concentration calculated from Lorenzen's (1967) equations.

Colonial cultures A1-3 and 165-7 were exposed to various UVB irradiances with constant PAR and UVA (see paragraph 2 above), and then extracted at 50°C for 30 min in the culture medium; 323 nm absorption was calculated per live cell to ascertain whether exposure to UVB influenced production of UV–absorbing compounds.

Xenic and axenic colonial strains of Phaeocystis antarctica A1-3, A1-4, DE10, and RG1.2 were also extracted and absorbance at 323 nm per unit chlorophyll a calculated. Absorbance per unit chlorophyll a was compared at 271 and 323 nm by a paired t-test. The value obtained for the A1-3 strain was also used to predict the attenuation of UVB radiation in Antarctic coastal waters.

3. RESULTS AND DISCUSSION

UV–absorbing substances are produced by various organisms, including eye lens tissue of fish (Dunlap et al. 1989), higher plants (Caldwell 1981), the cyanobacterium Nostoc commune (Scherer et al. 1988), corals (Dunlap et al. 1986), seaweeds (Sivalingam et al. 1974, Wood 1989, Karentz et al. 1991c) and phytoplankton (eg Sivalingam et al. 1974, Carreto et al. 1990, Karentz et al. 1991c). There is considerable evidence that these compounds shield the organisms from UV damage (eg. Caldwell 1981, Dunlap et al. 1989, Wood 1989, Karentz et al. 1991b, Vernet et al. 1994).

Aqueous concentrates of the colonial stage of Phaeocystis antarctica exhibited strong absorbance at 323 nm, a shouldered peak at 271 nm and a third peak at 211 nm (Fig. 2A). These absorbances greatly exceeded that of chlorophyll a at 680 nm and carotenoids. The colourless UVB absorbing compounds were fully soluble in water at 50°C, as evidenced by the lack of any absorbance in resuspended concentrate from this extraction. These UVB–absorbing compounds have also been found in field samples from Antarctic coastal waters (Davidson unpublished data).

Only the absorbance peak at 211 nm was present in aged axenic cultures in which no living cells remained (Fig. 2B). Thus, this peak represented the absorbance of the colony matrix

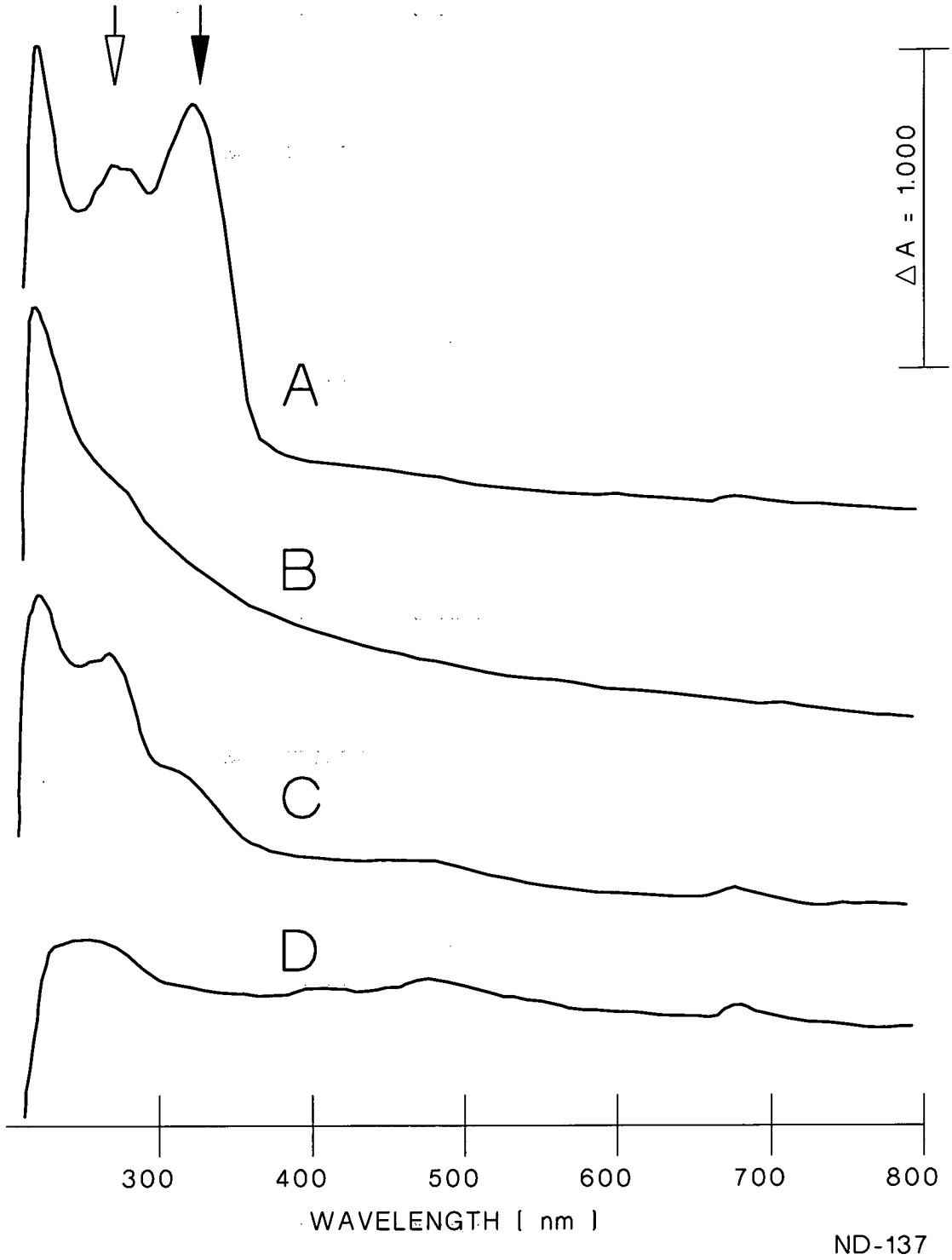


Fig. 2. Absorption spectra of cell concentrates from (A) colonies of an axenic isolate of Phaeocystis antarctica with a prominent peak at 323nm (closed arrow) and shouldered peak at 271 nm (open arrow), unmarked peak at 211 nm, and peak at 680 nm from chlorophyll a; (B) dead axenic colonies of Phaeocystis antarctica possessing only the 211 nm peak; (C) colonies from an East Australian Current Phaeocystis isolate; (D) axenic motile cells of P. antarctica.

which constituted most of the algal volume (Davidson & Marchant 1987) and any residual material from dead cells. Absorbance at wavelengths shorter than 280 nm is unlikely to have conferred protection because of the marked attenuation of such short wavelengths by the atmosphere and water (Smith & Baker 1979), but provided a background absorbance on which was superimposed that of the protective compounds. The disappearance of the 271 and 323 nm peaks from the aged axenic cultures showed that they were labile in the absence of bacteria. Absorption maxima were significantly higher at both 271 and 323 nm in late stationary–phase axenic cultures than those of the same strain and culture age containing bacteria ($0.025<P<0.050$, $n=8$), indicating that the alga was producing the compounds and that bacteria were involved in their decomposition. The ability of Phaeocystis antarctica to inhibit bacterial growth within the immediate vicinity of growing colonies (Sieburth 1960, Davidson & Marchant 1987) such that their number, in the order of 10^8 cells l^{-1} (approximately an order of magnitude less than that during stationary phase) would prolong retention of the UVB absorbing compounds by this life stage. That these compounds were labile and degraded by bacteria indicates that they are likely to be only short-lived outside growing colonies.

Phaeocystis from the East Australian Current could not be maintained in axenic culture. Thus, bacterial decomposition of the UV–absorbing compounds may contribute to the lower absorbance found in this strain (Fig. 2C). Bacterial impact was minimised, however, by using cultures in the exponential growth phase. Davidson and Marchant (1987) reported that bacterial numbers were more than an order of magnitude less in the mid–exponential growth than in the stationary phase. Cells of the flagellate stage in the life cycle of Antarctic strains contained negligible concentrations of these compounds (Fig. 2D). The absorbance at 323 nm of the media containing cultures of flagellates in log phase of growth was low (≤ 0.0034 cm^{-1}). Cultures of colonial Phaeocystis from near Tasmania, the North Sea and the English Channel, grown under identical conditions, in exponential growth phase, and having the same morphology, were similar to the East Australian Current material in having substantially less of the 323 nm absorbing material per μg of

chlorophyll a than the Antarctic strain (Table 1). The marked difference in the amount of UVB absorbing compounds is likely to reflect differences in Phaeocystis species (Medlin et al. 1994). However, the reason that P. antarctica contains much higher concentrations than cool temperate and northern hemisphere strains/species is uncertain.

Table 1. Absorbance at 323 nm of 50°C–extracted 323 nm absorbance per unit chlorophyll a of colonial Phaeocystis antarctica (A1-3), and Phaeocystis strains from Port Esperance, Tasmania (PE2), East Australian Current (165-7) and English Channel (Veldhuis & 540)

Strain	Absorbance
A1-3	0.1173
PE2	0.0195
165-7	0.0143
Veldhuis	0.0048
540	0.0047

Determination of both the proportion of undamaged cells following exposure to enhanced UV irradiation and their viability indicated that the UV–absorbing compounds provided substantial protection to colonial Phaeocystis antarctica cells. Viability studies showed that the live cell concentration, predicted from the growth rate of the unirradiated control, were well within the standard deviation of the observed cell concentration (Table 2). The exception, at an irradiance of 0.28 W m^{-2}, was probably due to the counting procedure. This was the only culture in which the observed growth rate was negative between Days 0 and 2, by which time the predicted value fell within the standard deviation of the observed cell concentration. Thus, the characterization of chlorotic and greatly vesicularized cells as "dead" in the survival studies shown in Fig. 3 provided valid criteria by which to quantitate the viable cells in culture after UVB irradiation.

The efficacy of the UV–absorbing compounds in protecting colonial Phaeocystis cells from UV radiation is apparent from Fig. 3. The percentage of undamaged (live) cells in cultures of colonial East Australian Current and Phaeocystis antarctica motile cells decreased markedly with increasing total irradiance (Fig. 3A). In contrast, colonial P. antarctica cells were apparently unaffected when the total irradiance was lower than 13.27 W m^{-2}, corresponding to an experimental UVB irradiance of 0.32 W m^{-2}. This irradiance is approximately 60% of peak and 150% of average surface irradiance measured at the Australian Antarctic station of Casey at Latitude 67°S (Wood WF 1991, pers. comm.). At higher irradiances, mortality of colonial cells increased at about the same rate as motile cells. The percentage of live East Australian Current colonial cells was equivalent at each UVB irradiance, irrespective of whether this was under increasing total radiation (Fig. 3A) or UVB alone (Fig. 3B). This contrasted with the colonial strain of P. antarctica. Under increasing total irradiance the proportion of live cells declined to approx 40% when exposed to a total irradiance of 24.5 W m^{-2}, of which 5.81 x 10^{-1} W m^{-2} was UVB (Fig. 3A). Increasing UVB irradiance alone produced no significant mortality until irradiance exceeded 0.60 W m^{-2} (Fig. 3B), suggesting inhibition by elevated PAR under increasing total irradiance, or sustained greater cell damage since the UVB spectrum was only attenuated to Distribution 6 (Fig. 1) in all cases. This UVB irradiance approximates peak Antarctic coastal surface irradiance (Wood WF 1991 pers. comm.).

Table 2. Test of viability using growth rate of colonial cells. Growth rate of unirradiated control was 0.22 doublings per day; concentration of organisms is cells x 10^{-3} ml^{-1}±SD

UVB Irradiance (x10^{-1} W.m^{-2})	Observed Live Cells at Day 0	Observed Live Cells at Day 13	Predicted Viable Cells at Day 0
0 (Control)	17 ± 6.7	111 ± 62.3	-
0.286	51 ± 25.2	99 ± 28.1	14.1
0.598	29 ± 7.8	184 ± 109.2	26.1
1.310	14 ± 13.7	78 ± 34.3	11.1
2.059	0.3±0.5	3 ± 2.76	0.5

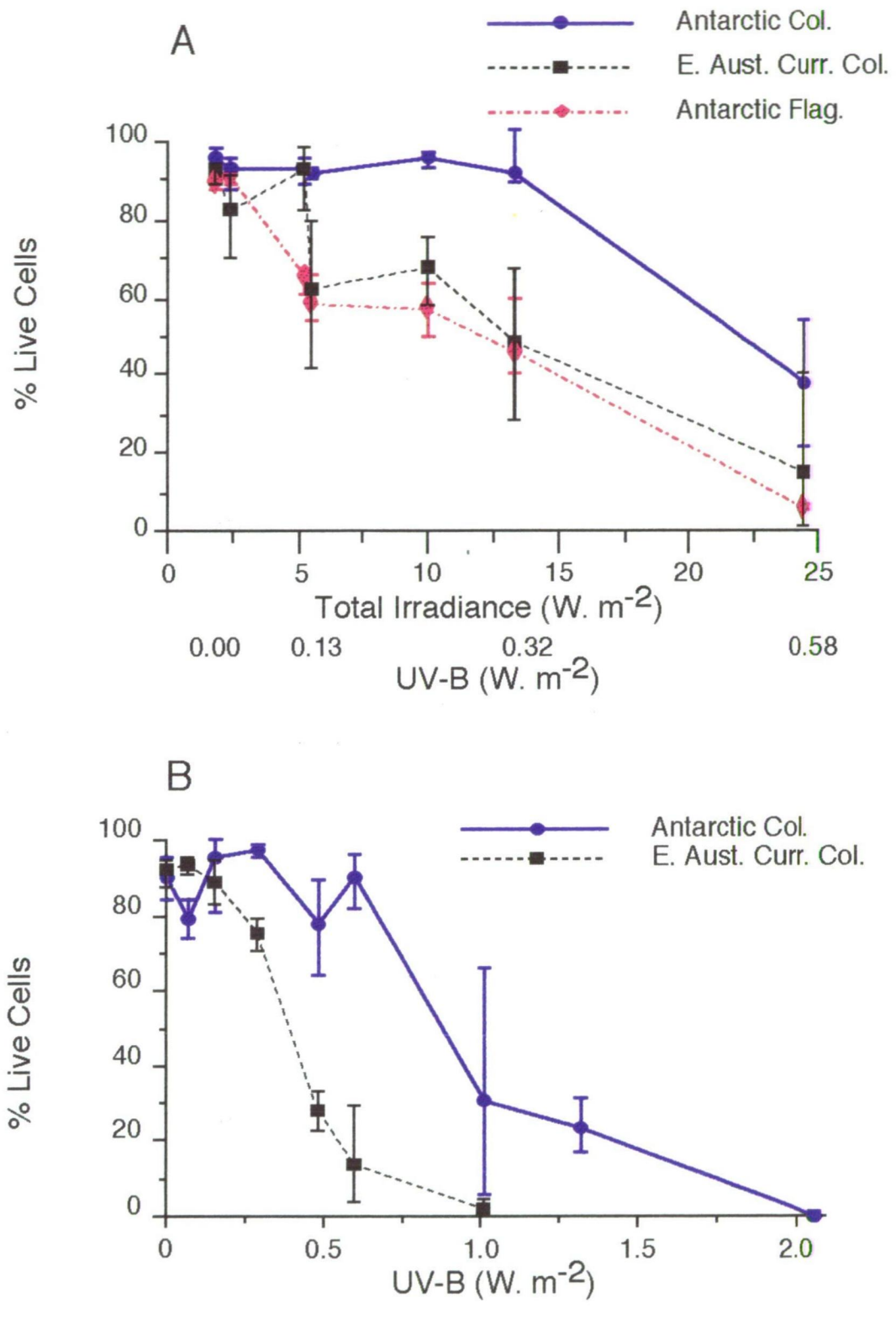


Fig. 3. (A) Percentage of live cells of East Australian Current colonial (E. Aust. Curr. Col.), Phaeocystis antarctica colonial (Antarctic Col.) and flagellate (Antarctic Flag.) cultures of Phaeocystis exposed to increasing light intensities provided by a Suntest UV/white light source; and (B) in Antarctic and East Australian Current colonial cultures irradiated with various intensities of UVB and constant photosynthetically active radiation (PAR) and UVA. Error bars represent standard errors calculated after Zar (1984).

Production of UV protecting pigments is enhanced by exposure to UV radiation in higher plants (Caldwell 1981) and the cyanobacterium Nostoc commune (Scherer et al. 1988). Sublethal irradiance of colonial Phaeocystis antarctica with UVB was also found to significantly increase the absorbance per cell at 323 nm (Fig. 4). Only three of the experimental irradiances were sublethal to East Australian Current colonial Phaeocystis, but these appeared to reduce rather than promote pigment production. Thus, the rate of UV–absorbing compound production by the colonial stage of P. antarctica is responsive to ambient UVB climate. The data showed that the resulting increase in UVB absorbance by the P. antarctica would enhance their survival. Should the UVB screening prove insufficient, cells were damaged or died and production rates of UVB-absorbing compounds would decline, increasing the vulnerability of the reminder of the population to further UVB damage. However, the colonial stage of P. antarctica carried a substantial complement of UV–absorbing material irrespective of past UV climate (Fig. 2A) which would help mitigate such breakdown of UVB protection in the wild. In contrast, the East Australian Current colonial Phaeocystis strain produced less pigment and was apparently unable to increase the rate of production under UVB stress.

The absorption of colonial Phaeocystis antarctica Strain A1-3 at 323 nm was 1.219 cm^{-1} at a chlorophyll concentration of 810 $\mu g\ l^{-1}$. In the marginal ice edge zone near the Antarctic coast, the concentration of colonial stage P. antarctica reached 6×10^7 cells l^{-1}, which corresponds to a chlorophyll a concentration of 4.59 $\mu g\ l^{-1}$ (Davidson & Marchant 1992a). Relating the chlorophyll a normalized absorbance in culture to the chlorophyll a concentration of an Antarctic P. antarctica bloom indicates that, at such a concentration, absorbance would be about 80% m^{-1} at 323 nm. Although this predicted value is only approximate (combining data from different laboratory and field studies), it does indicate that P. antarctica is likely to protect other organisms present in the waters column at the same time against UVB.

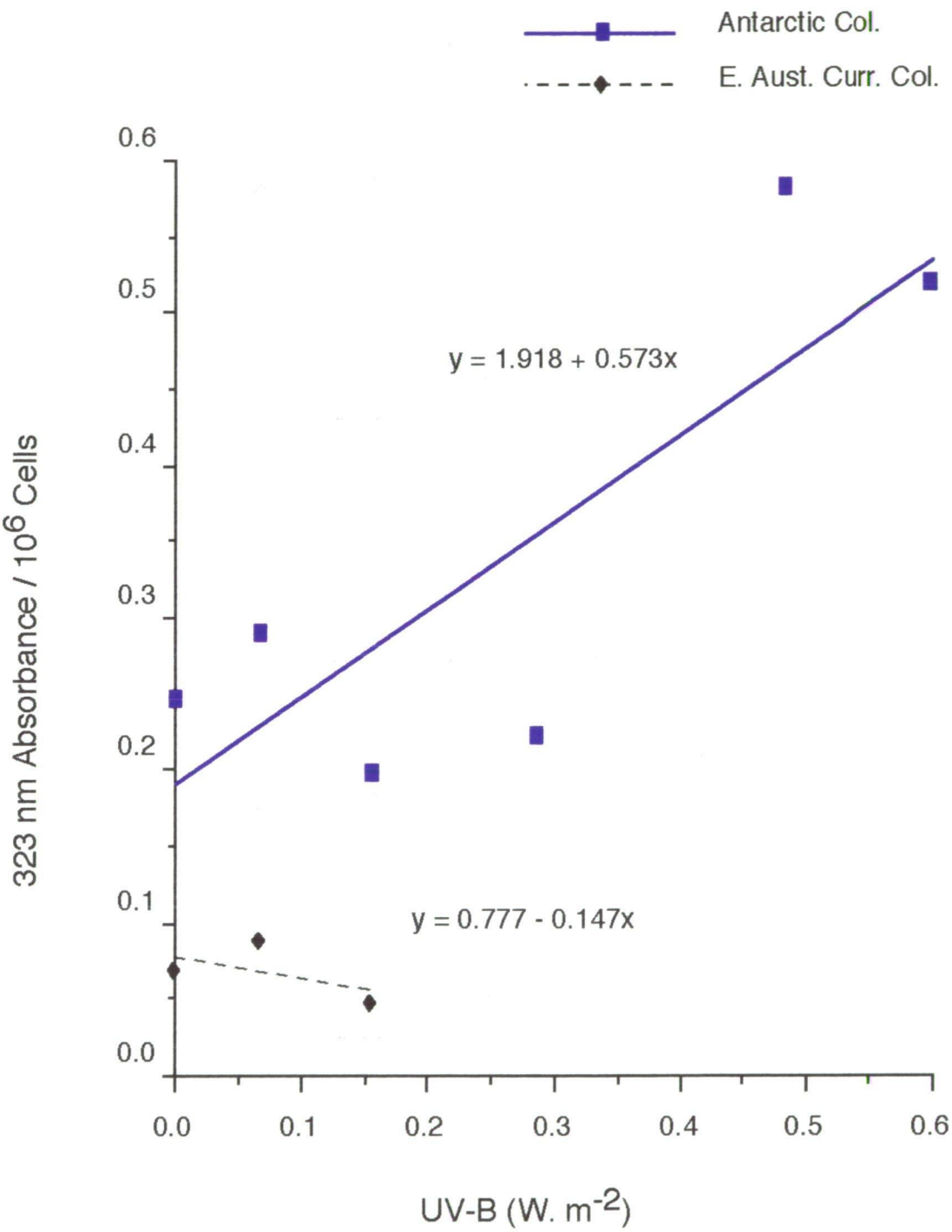


Fig. 4. The UVB absorbing pigment per cell plotted against sublethal UVB irradiance at constant PAR and UVA. Regression for P. antarctica (Antarctic Col.) was significant ($0.02<P<0.05$), while that for East Australian Current Colonial Phaeocystis (E. Aust. Curr. Col.) was not.

Vernet et al. (1994) correlated increased in situ UV–absorption with elevated concentrations of 19'-hexanoyloxyfucoxanthin; a pigment which, in Antarctic waters, strongly indicates the presence of Phaeocystis antarctica (Davidson & Marchant 1992b). Subtidal and intertidal Antarctic fish, invertebrates and algae also commonly contain UV–absorbing mycosporine amino acids (Karentz et al. 1991b). While numerous authors report that natural Antarctic plankton contain UV–absorbing substances, these studies do not identify the species responsible for this absorbance (Mitchell et al. 1989, Vernet et al. 1989, Gieskes & Kraay 1990). Thus, the extent to which key Antarctic phytoplankton species possess UV–absorbing compounds is largely unknown. Still less is known about the UVB photobiology of these key species. The following chapter examines UV–absorption, survival and growth amongst eight commonly occurring species of Antarctic marine diatoms exposed to a range of UVB irradiances. Results are compared with those for Phaeocystis antarctica.

CHAPTER 6

Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms

1. INTRODUCTION

The effect of increasing solar UVB flux on Antarctic phytoplankton and higher trophic levels is equivocal (El-Sayed et al. 1990, Karentz 1991, Villafãne et al. 1995, Davidson et al. 1996 see also Chapter 1 section 6.1.2 and 6.2). Calkins and Thordardottir (1980) found that the tolerance of six high latitude marine diatoms to UVB was similar and concluded that most organisms would adapt to enhanced solar UV through increased protective pigmentation, repair, or avoidance mechanisms. Other authors have proposed a shift in species composition, favouring those species with greater tolerance of UVB (Häder & Worrest 1991, Karentz 1991, Marchant & Davidson 1991, Vincent & Roy 1993, Davidson et al. in press). The mechanisms of UVB protection in Antarctic phytoplankton species are largely unknown, although UV–absorbing compounds and DNA repair mechanisms have been reported for some species (Karentz 1988, Bidigare 1989, Mitchell et al. 1989, Marchant et al. 1991, Karentz et al. 1991a, c).

Phytoplankton form the base of the Antarctic food web and sustain the wealth of life for which the Southern Ocean is renown (Ainley et al. 1986). Information on the effects of UVB on growth and survival of Antarctic marine phytoplankton is integral to understanding the impact of elevated UVB exposure on the Southern Ocean ecosystem. Species specific investigations on the impact of UVB are necessary to predict the effect of ozone depletion on these primary producers. This chapter examines the effect of UVB exposure on the growth rates, survival and UV–absorption of five species of Antarctic marine diatoms. Their UVB tolerance is compared with that of Phaeocystis antarctica.

2. MATERIALS AND METHODS

2.1. Light measurements

All measurements of irradiance were made with an International Light IL 1700 Radiometer equipped with detectors to measure photosynthetically active radiation (PAR), UVA (320 to 400 nm) and UVB (see Chapter 5, Fig 1). A National Institute of Standards and Technology intercomparison package (NIST Test #534/240436-88) was used to calibrate each light sensor.

2.2. Cell isolation and culture

Unialgal cultures of the diatoms Nitzschia lecointei V. H., Proboscia (Rhizosolenia) alata (Brightwell) Sundström, Proboscia (Rhizosolenia) inermis (Castracane) Jordan and Ligowski, Thalassiosira tumida (Jan.) Hasle, Stellarima (Coscinodiscus) microtrias (Ehrenberg) Hasle and Sims, Odontella weisflogii (Jan.) Grunow, Fragilariopsis curta (V. H.) Hasle and Chaetoceros simplex Ostenfeld were isolated from sea ice collected in Prydz Bay, Antarctica during the 1990/91 austral summer. Cultures were maintained in 250 ml glass flasks using f/2 growth medium (Guillard & Ryther 1962) at a temperature of 4 ± 2°C. Cool white fluorescent lights provided photosynthetically active radiation (PAR) intensity of 11.80 $J.m^{-2}.s^{-1}$ (58.85 $\mu E.m^{-2}.s^{-1}$), with no UVB enhancement, on a 12 h light : 12 h dark cycle.

2.3. UVB enhanced treatments

50 ml Lux tissue culture flasks (which completely absorbed wavelengths below 295 nm) were filled from a single parental culture in exponential growth phase and irradiated for 24 hours in a 48 hour experimental period (6 h light : 12 h dark : 12 h light : 12 h dark : 6 h light). Day 0 in data calculations occurs at the end of this irradiance period. Exposures were conducted in a Thermoline controlled environment cabinet at 4 ± 2 °C with cool white fluorescent tubes to provide PAR and UVA (320 - 400 nm), with UVB provided by FS20T 12 UVB Westinghouse sunlamps. PAR and UVA irradiances were 12.13 ± 2.13 $W.m^{-2}$ (60.5 ± 10.4 $\mu E.m^{-2}.s^{-1}$) and 1.19 ± 0.68 $W.m^{-2}$ respectively. The spectral

distribution and UVB irradiance were varied by attenuation with glass filters (Marchant et al. 1991). The zero UVB irradiance treatment was screened by mylar which excluded light below 320 nm. Sensors were covered by each attenuating glass screen and a single layer of Lux culture flask to measure the experimental irradiances to which the diatoms were exposed. UVB irradiances of 0.10 to 3.40 $W.m^{-2}$ were chosen to span the range of 20% to 650% of peak UVB exposure as measured at an Antarctic coastal site (Casey station, 66°S) in the 1989 summer (C. Roy, unpublished data). A single culture of each species was incubated at each of these irradiances and a control culture of each species was returned to culture maintenance conditions and received only PAR.

2.4. Calculation of viable cell concentration

Immediately after irradiation (Day 0) 5 or 10 ml (depending on cell concentration) of the control culture was sedimented with Lugol's iodine and the concentration of cells with cytoplasmic contents (live cells) was calculated from counts over 15 replicate fields, using an Utermöhl settling tube and inverted microscope. The mean cell concentration in the control culture at Day 0 was then calculated ($N_{0\ control}$). Also on Day 0, a 5 ml aliquot of each irradiated culture and the control were inoculated into 30 ml of f/2 medium in a glass flask and returned to the culture maintenance conditions described previously. These subcultures were incubated for up to 10 days and the concentration of live cells counted at 2 to 4 day intervals depending on their growth rate. The growth rate of the control culture of each species (K control) was calculated using the equation of Verity et al. (1988a) (equation 1). Equation 2 was then used to calculate the viable cell concentration on Day 0 (N_0 irradiated) using the cell concentration for each of the 15 replicate fields after ongrowth (N_t) the growth rate of the control (K control) and the time of culture ongrowth (t)

$$K = 1/t \times \log_2 \frac{N_t}{N_0} \quad (1)$$

$$N_0 \text{ irradiated} = \frac{N_t \text{ irradiated}}{{}_2K \text{ control} \times t} \quad (2)$$

$$S\% = \frac{N_0 \text{ irradiated}}{N_0 \text{ control}} \times 100 \qquad (3)$$

where K = growth rate, t = number of days of growth, N_t = number of cells at time t, N_0= number of cells immediately after irradiation (Day 0) and S% = percent survival.

The calculated viable cell concentration of each replicate field at Day 0 (N_0 irradiated) was then converted to percent survival (S%) in comparison with the unirradiated control at Day 0 (N_0 control) using equation 3. In cases where the cell number in irradiated cultures was greater than that in the control culture, computed survival could not exceed 100 % . The percent survival in each replicate field was arcsine square root transformed, the mean and standard error of the replicate fields computed and the mean and upper and lower confidence intervals sine squared to revert the data to percentages (Zar 1984).

Equation 1 was then used to calculate the growth rate of all ongrown irradiated cultures. Growth rates were calculated for each species from the day at which the cell concentration in culture had reached a sufficient concentration to allow statistically acceptable mean estimates (N_0) and the concentration four days later (N_t). t_0 for each species were:- Nitzschia lecointei - Day 4, Proboscia alata - Day 6, Proboscia inermis - Day 6, Thalassiosira tumida - Day 8, Stellarima microtrias - Day 8.

2.5. Removal of dark period from irradiance cycle

Nitzschia lecointei and Stellarima microtrias were exposed to the same experimental light irradiances as described above but with the dark period of the cycle removed, giving 24 hours of constant illumination. These two species were chosen as they exhibited different responses to treatments which included a dark period. The same procedures were followed as described above for determining survival and growth rate.

2.6. Measurement of UV absorption

A known volume of culture was filtered through 2.5 cm diameter Whatman GF/F filters. Filters were cut up into an homogeniser and 1.5 ml of 4 : 1, methanol : tetrahydrofuran

(MeTHF) added. The sample was then homogenised using a glass tube and teflon grinder for 30 seconds at approximately 1000 rpm and decanted into a centrifuge tube. A further 0.5 ml of MeTHF was added to rinse the homogeniser, this was again decanted into the centrifuge tube and the sample centrifuged at 480 x g for 10 min at 0°C. The absorbance of the supernatant was measured between 250 and 800 nm using a Hewlett Packard 8450A spectrophotometer. If measurements were not carried out immediately the extracts were stored at -120°C for no more than 4 weeks. The wavelength of maximum UV absorbance was identified and the peak absorption height above the adjacent minima measured for each extract. Data were then averaged over all cultures that received sublethal irradiances. Average absorbance was then normalised to chlorophyll a peak height at 665 nm. Cell carbon content was calculated for each species using cell concentration, volume and carbon conversion equations of Eppley et al. (1970) and the absorbance normalised to cell carbon concentration (C). The amount of UV–absorbing pigment was calculated per unit cell C to allow comparison between species that varied in volume from around 7.90 x 10^2 μm^3 to 1.92 x 10^5 μm^3 for Nitzschia lecointei and Proboscia inermis respectively. UV absorbance was also normalised to cell concentration. Regression analysis of log absorbance per cell for each species was used to ascertain whether the concentration of UV–absorbing compounds was promoted by increased UVB irradiance.

Absorption by UVB pigments, extracted cell contents and frustules was measured in exponentially growing cultures of Nitzschia lecointei, Proboscia alata, Thalassiosira tumida, Odontella weisflogii , Fragilariopsis curta, and Chaetoceros simplex, grown in f/2 medium under culture maintenance conditions (as above). Seven hundred ml of each culture was centrifuged at 200 x g for 40 mins at 0°C to concentrate the cells and the supernatant discarded. Two ml of 4 : 1, MeTHF was then added, the cells resuspended and the intracellular pigments allowed to extract overnight at 0 °C. The centrifugation was repeated and the absorption of the supernatant measured as above. To remove any contamination from intracellular UV–absorbing compounds, the extracted material was

rinsed 3 times by addition of 2.0 ml of MeTHF, resuspension and centrifugation at 200 g for 10 min at 0°C. The material was then resuspended in a further 2.0 ml of MeTHF and the absorbance measured as above.

To clear diatom frustules of organic contents, a known volume of the above MeTHF extracted cell concentrate was centrifuged at 480 x g for 10 mins and the MeTHF supernatant discarded. The sample was then digested for 24 hours in 5 ml of 30% H_2O_2 and 25g of $K_2Cr_2O_7$ added to oxidise and clean the frustules. The solution was diluted to 15 ml with Milli Q water, centrifuged at 480 x g for 1 hour and the supernatant again discarded. Microscopic examination of samples showed that this was sufficient to remove the cell contents from all species except Thalassiosira tumida; two treatments were necessary to clear the frustules of this species. Samples were resuspended twice in 15 ml of Milli Q centrifuged at 480 x g for 1 hour and the supernatant discarded. Finally, the cleared frustules were resuspended in a volume of MeTHF equal to that of the initial MeTHF extract and the absorbance measured (as above).

3. RESULTS

3.1. UVB absorbance

Only compounds with absorption >290 nm were investigated. Pigment extracts of Nitzschia lecointei, Proboscia alata, Proboscia inermis, Thalassiosira tumida, Stellarima microtrias and Fragilariopsis curta had chlorophyll a absorbance peaks at 665 nm and chlorophylls and carotenoids at around 440 nm (Fig. 1). None of the species investigated had any pronounced absorbance peaks in the UVB region of the spectrum. There was, however, increasing background absorption in the UV region of the spectrum and distinct absorbance peaks between 325 and 342 nm for each species (Table 1), the shoulder of which absorbed at UVB wavelengths (Fig. 1). The ratio of UV–absorbing compound peak height to that of chlorophyll a at 665 nm for the diatoms is 2.1:1 or less. Most of the absorption was at UVA wavelengths and absorption in the UVB region by the shoulder

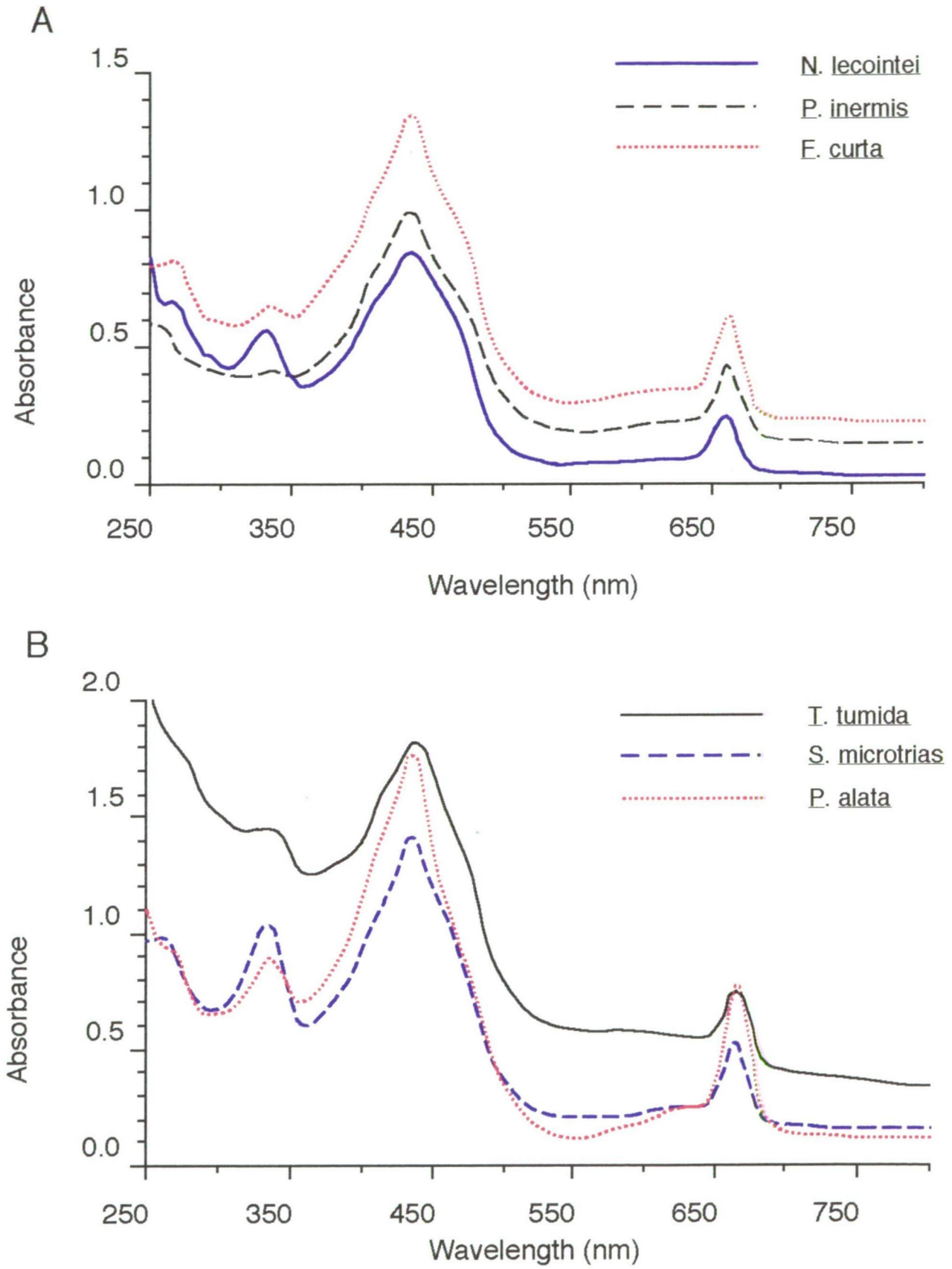


Fig. 1. Absorbance spectra of extracts in 80% methanol : 20 tetrahydrofuran between 250 and 800 nm for control cultures of (A) Proboscia inermis, Nitzschia lecointei and Fragilariopsis curta and (B) Thalassiosira tumida, Proboscia alata and Stellarima microtrias.

of these peaks was much less.

Table 1. UV–absorbing compounds in Antarctic marine diatoms and Phaeocystis giving wavelength of peak UV absorbance, the ratio of UV–absorbing compound peak height to chlorophyll a peak height at 665 nm and the UV–absorbance per µg cell carbon. * value calculated from data in Marchant et al. (1991). Data presented are mean values of all sublethal irradiances

Species	Peak absorbance (nm)	UV Abs : chl a ratio	UV Abs / µg cell C
Nitzschia lecointei	325	0.9	1.10×10^{-6}
Proboscia alata	336	1.7	6.86×10^{-5}
Proboscia inermis	340	2.1	6.17×10^{-5}
Thalassiosira tumida	342	1.2	5.08×10^{-5}
Stellarima microtrias	342	1.8	5.91×10^{-7}
*Phaeocystis cf. pouchetii	323	27.5	1.04×10^{-2}

As the concentration of chlorophyll a changes in response to UVB exposure (Bidigare 1989) it was not used in normalising UVB-induced changes. Log absorbance per unit cell C for each species over the range of UVB irradiances is shown in Fig. 2. Data from Phaeocystis antarctica (Marchant et al. 1991) using similar methods is included for comparison. Regression analysis of the UV absorbance peak height per cell against sub-lethal irradiance showed that increased UVB flux elicited no significant response in UV absorbance in any diatom and the F-test shows that the regression slopes were not significantly different from zero (Table 2).

Absorption of MeTHF insoluble material by Nitzschia lecointei, Proboscia alata, Odontella weisflogii, Fragilariopsis curta and Chaetoceros simplex (Fig. 3A-B, D-F) gradually decreased with increasing wavelength while that of T. tumida remained approximately constant (Fig. 3C). Absorption by cleared frustules of each species also decreased with increasing wavelength but only accounted for between 13 and 29% of the

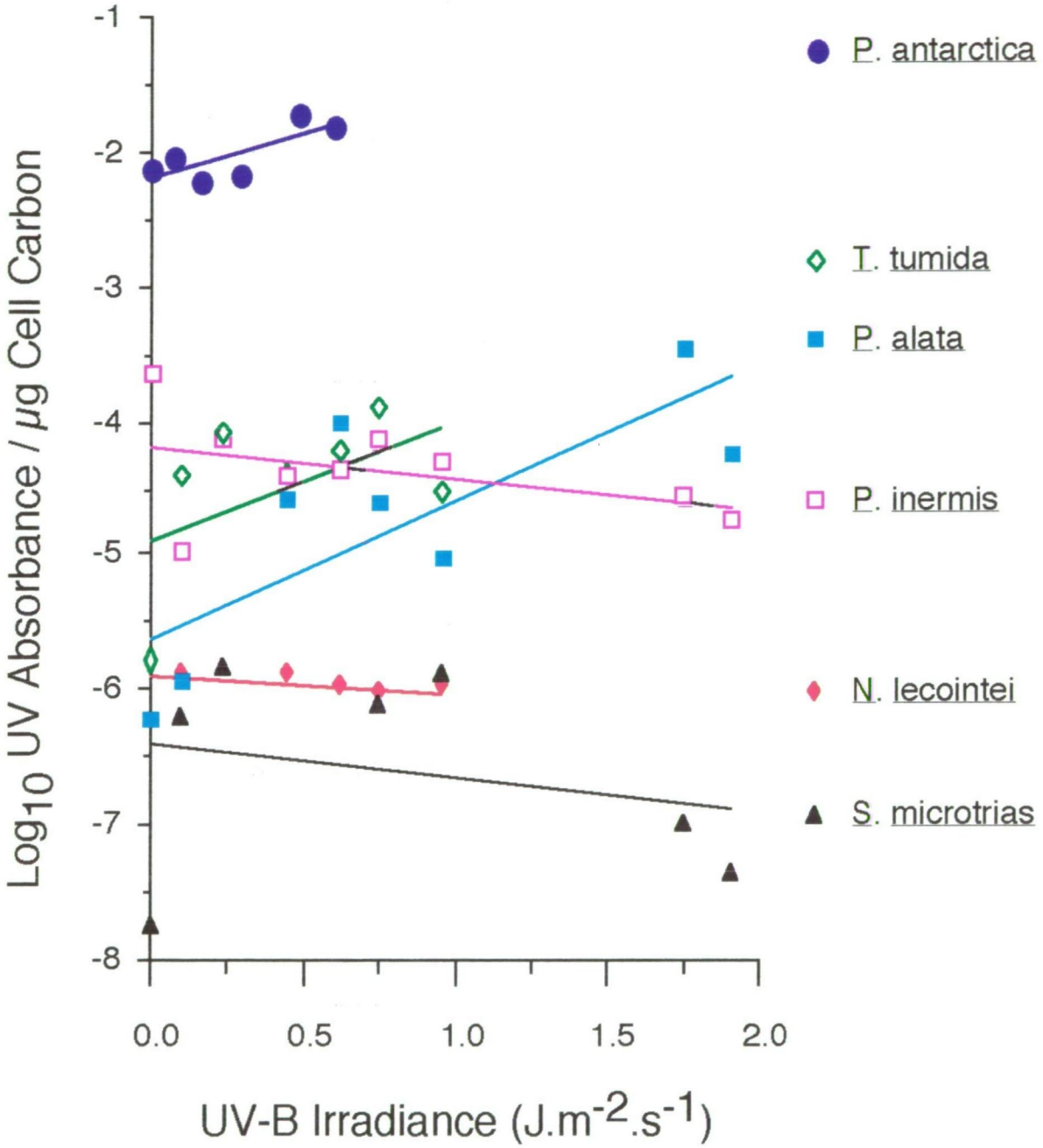


Fig. 2. Log peak UV absorbance per unit cell carbon of Proboscia inermis, Nitzschia lecointei, Thalassiosira tumida, Proboscia alata, Stellarima microtrias and Phaeocystis antarctica* against sub-lethal UV-B irradiances. * Data from Marchant et al. (1991).

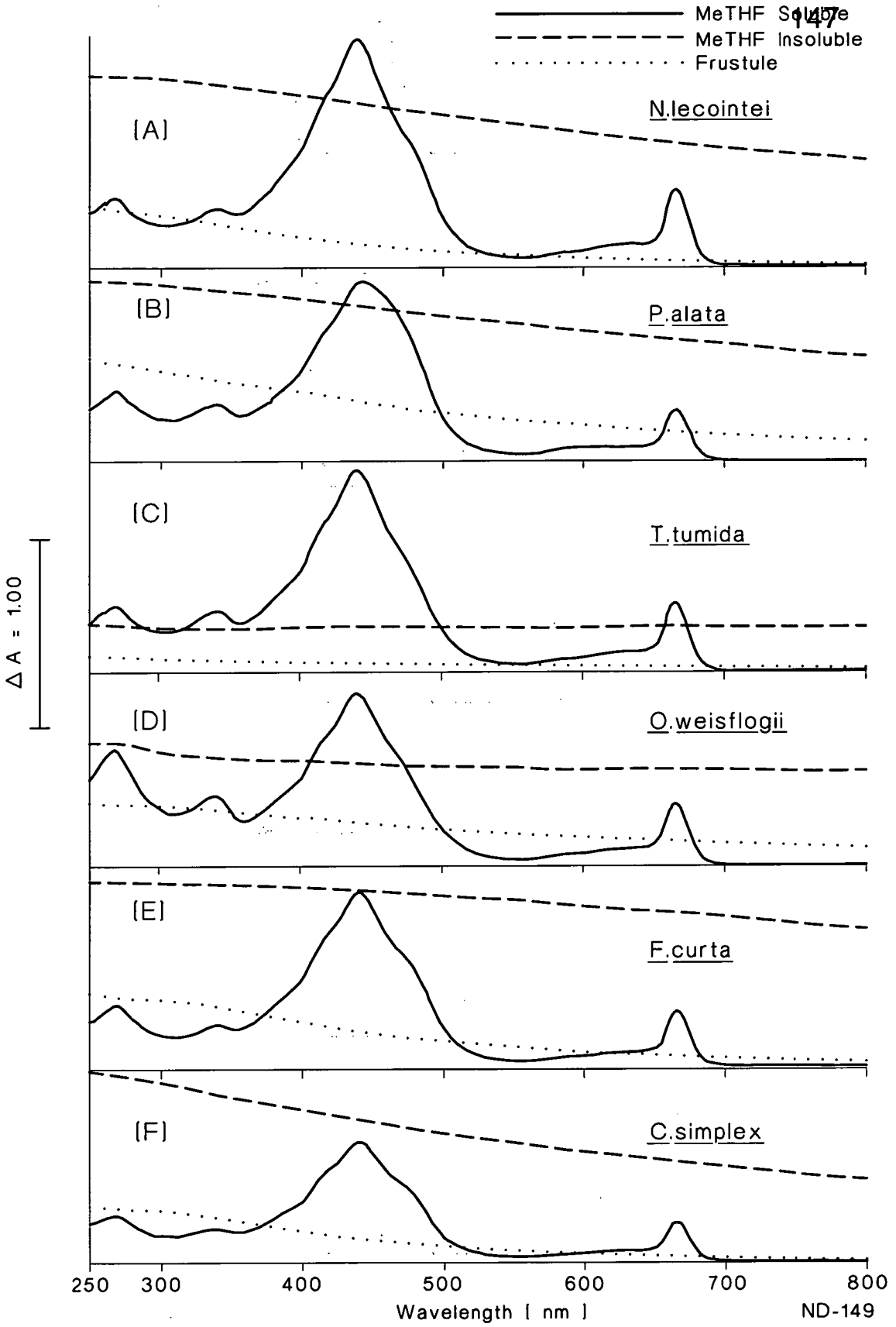


Fig. 3. Absorbance spectra of extracts in 4 methanol : 1 tetrahydrofuran (MeTHF) for cultures of (A) Nitzschia lecointei, (B) Proboscia alata, (C) Thalassiosira tumida, (D) Odontella weisflogii (E) Fragilariopsis curta and (F) Chaetoceros simplex grown under maintenance conditions. Cells extracted with MeTHF and the insoluble material oxidised to clear the frustules. Absorbance by MeTHF soluble compounds (MeTHF Soluble), MeTHF insoluble matter (MeTHF Insoluble), and cleared frustules (Frustles) were measured between 200 and 800 nm.

total UVB absorption by the cells (Fig. 3 & 4). Total cellular UVB absorption per µg cell carbon varied between 3.4 x 10^{-5} for T. tumida to 8.2 x 10^{-4} for N. lecointei (Fig. 4). MeTHF–soluble pigments comprised between 12 and 26% of this absorption. The exception was T. tumida, where it accounted for 45 % of UVB absorption. The majority of UVB absorption was due to MeTHF insoluble cell contents except for T. tumida were the proportion was slightly less than that of the MeTHF soluble material (Fig. 4).

Table 2. Regression statistics obtained in linear regression of UV absorbance per cell against sublethal UV-B irradiance for Antarctic marine diatoms.

Species	P{r}	P{F}
Nitzschia lecointei	.2>x>.1	.1955
Proboscia alata	.2>x>.1	.1009
Proboscia inermis	.5>x>.2	.2732
Thalassiosira tumida	.5>x>.2	.2542
Stellarima microtrias	>0.50	.5851

3.2. UVB response - survival and growth rate

Survival of diatoms exposed to UVB differed between species (Fig. 5). Diatoms screened with Mylar received no UVB (irradiance 0, Fig. 5) but received unattenuated UVA and exhibited low survival (9 - 32%). Survival of Nitzschia lecointei, Proboscia alata and Proboscia inermis at sublethal irradiances approximated 100 % survival (Fig. 5 A - C) but that of Thalassiosira tumida and Stellarima microtrias at sublethal irradiances ranged from 51 to 85 % and 59 to 75 % respectively (Fig. 5D, E). At 1.75 J.m^{-2} s^{-1} the survival of N. lecointei, and P. alata fell to 17 and 14% respectively and survival was negligible at 3.4 J.m^{-2} s^{-1}(Fig. 5A, B). No significant change in survival of P. inermis, T. tumida and S. microtrias occurred until a UVB irradiance of 3.4 J.m^{-2} s^{-1} at which irradiance their survival fell to 25% (Fig. 5C, D, E). In contrast, the survival of Antarctic colonial Phaeocystis antarctica (Fig. 5F) was reduced to 30% at a UVB irradiance of 1.0

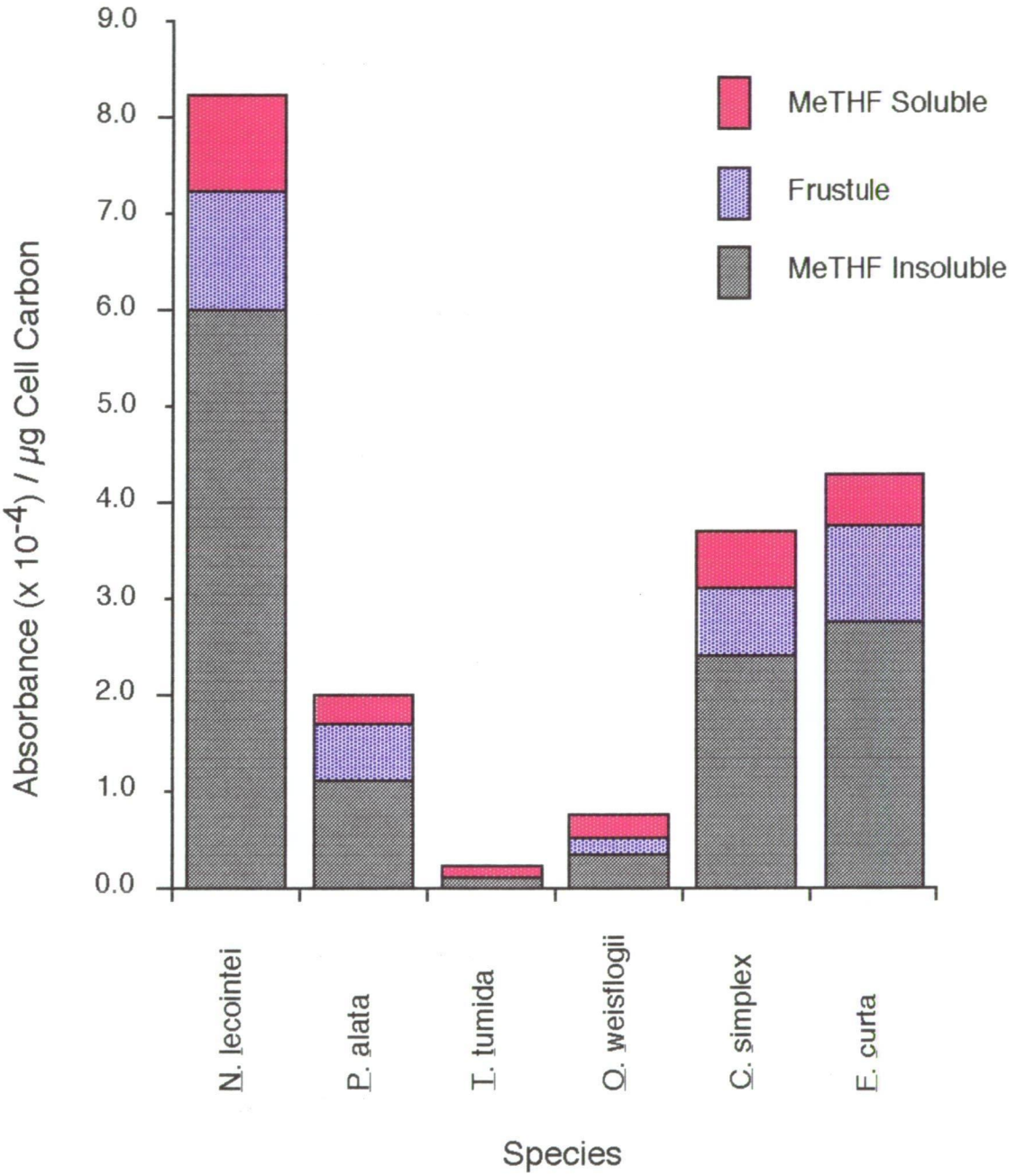


Fig. 4. Average UV-B (280 - 320 nm) absorbance of MeTHF soluble compounds (MeTHF Soluble), MeTHF insoluble matter (MeTHF Insoluble) and cleared frustules (Frustules) per μg cell carbon for Nitzschia lecointei, Proboscia alata, Thalassiosira tumida, Odontella weisflogii, Chaetoceros simplex and Fragilariopsis curta.

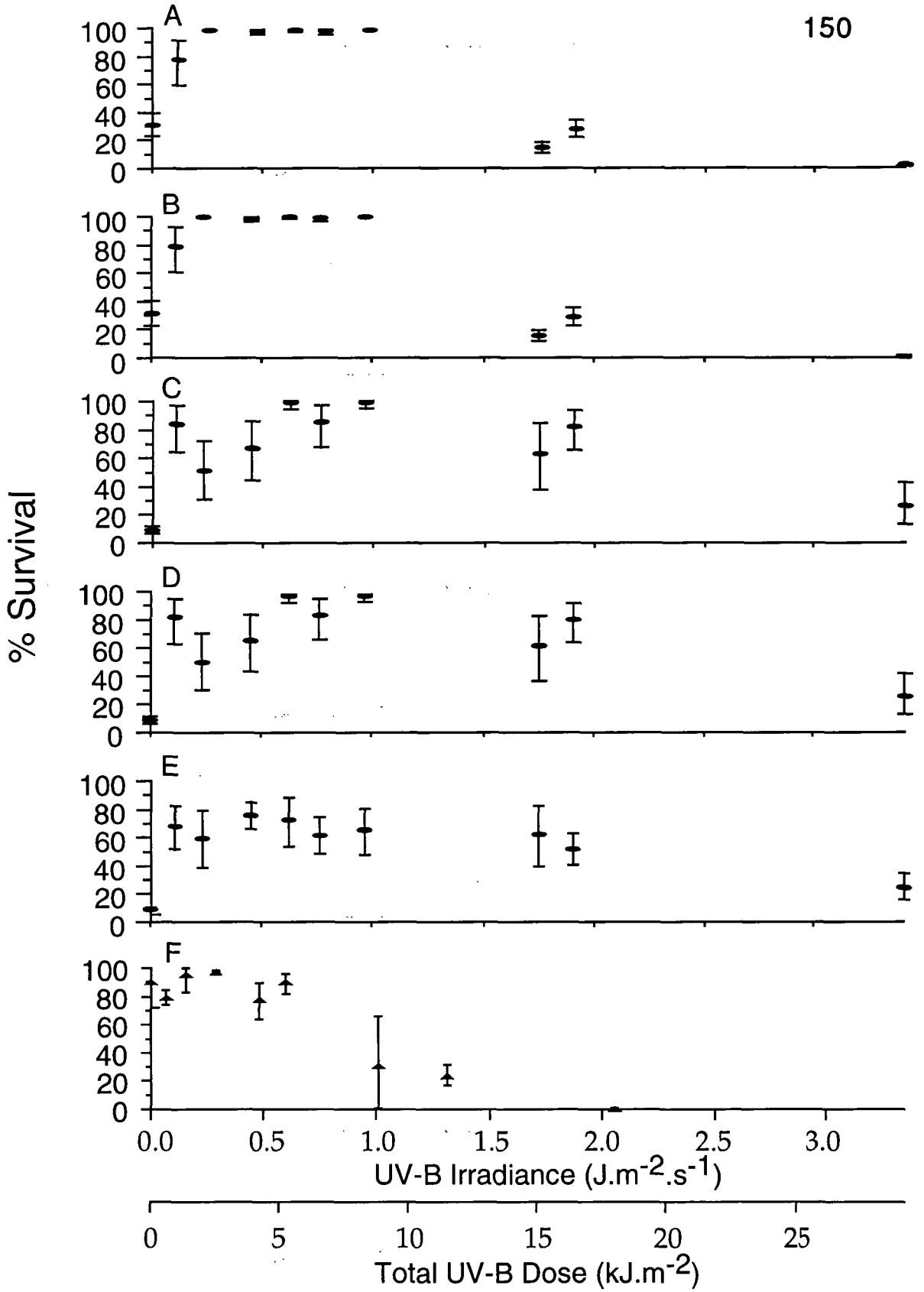


Fig. 5. Percent survival of (A) Nitzschia lecointei, (B) Proboscia alata, (C) Proboscia inermis, (D) Thalassiosira tumida, (E) Stellarima microtrias and (F) Phaeocystis antarctica* irradiated for 24 in 48 h against UV-B irradiance. * Data from Marchant et al. (1991). Error bars represent standard errors calculated from Zar (1984).

$J.m^{-2}\ s^{-1}$ with survival reduced to 0% at a UVB irradiance of 2.1 $J.m^{-2}\ s^{-1}$ (Marchant et al. 1991).

Regression analysis showed that there was no significant relationship between the growth rate after irradiation of the diatoms and the UVB irradiance they received (Table 3). The growth rate of Nitzschia lecointei appeared to decline as UVB irradiance increased and this was not observed until the highest irradiance (3.4 $J.m^{-2}\ s^{-1}$; Fig. 6). Growth rates for irradiated cultures of Proboscia alata, Proboscia inermis, Thalassiosira tumida, and Stellarima microtrias were comparable to those occurring in the PAR control.

Table 3. Regression statistics obtained in linear regression of post-irradiation growth rate against UV-B irradiance for Antarctic marine diatoms.

Species	P{r}
Nitzschia lecointei	0.1<x<0.05
Proboscia alata	0.2<x<0.1
Proboscia inermis	0.1<x<0.05
Thalassiosira tumida	0.5<x<0.2
Stellarima microtrias	0.2<x<0.1

3.3. Dark period removal

Removal of the dark period from the irradiance of both Nitzschia lecointei and Stellarima microtrias elicited a different survival response (Fig. 7) from those treatments incorporating a dark period. N. lecointei cells survived an irradiance incorporating dark periods of 1.75 $J.m^{-2}\ s^{-1}$ (Fig. 5A) but survived all but the maximum irradiance when exposed without dark periods (Fig. 7A). In contrast with N. lecointei, survival of S. microtrias during irradiation including a dark period did not decline significantly until receiving 3.2 $J.m^{-2}\ s^{-1}$. When the dark period was removed, survival of S. microtrias declining to 23% at an irradiance of 1.75 $J.m^{-2}\ s^{-1}$. In addition, survival of S. microtrias

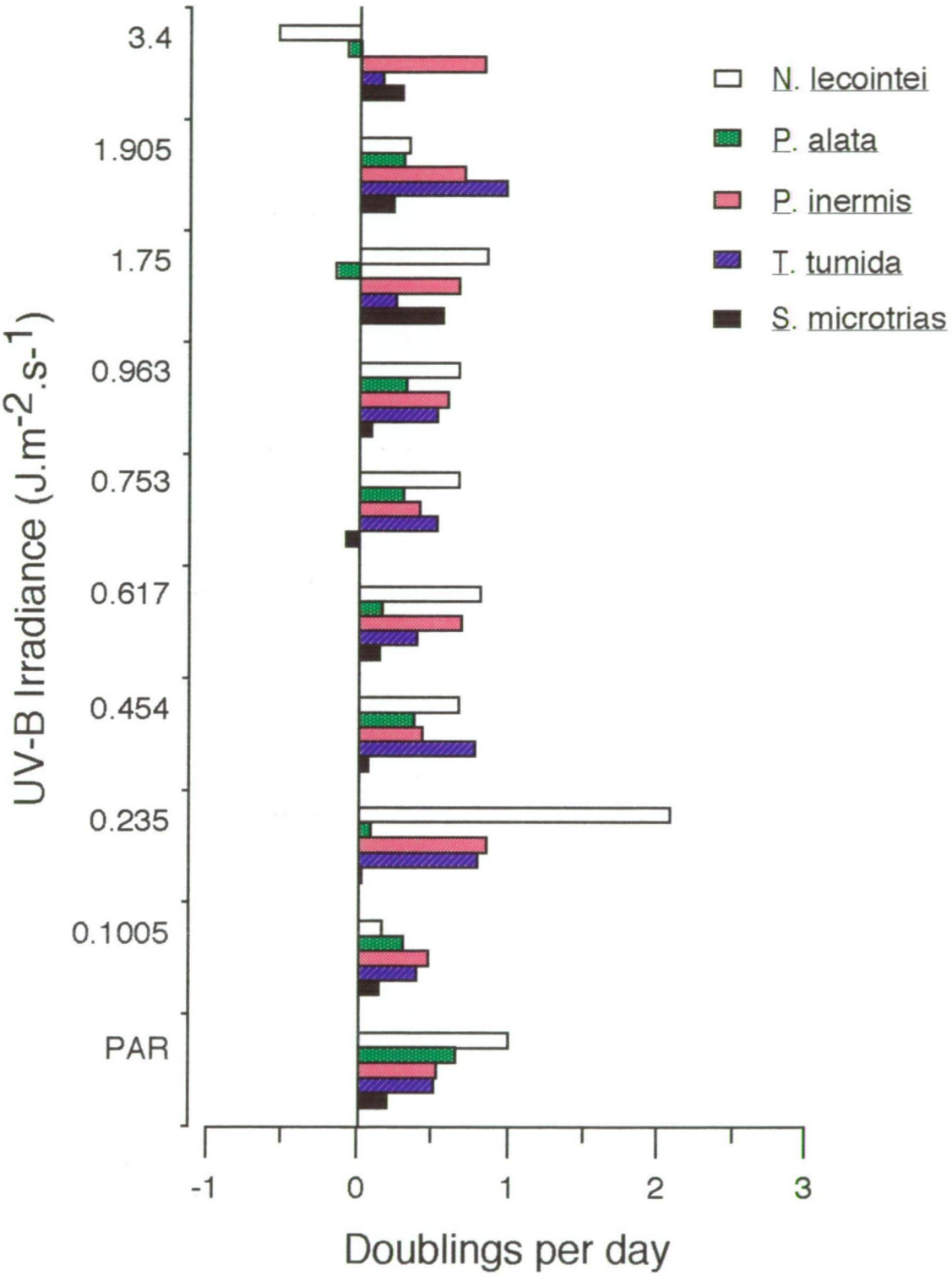


Fig. 6. Growth rate of exponentially growing cultures of Nitzschia lecointei, Proboscia alata, Proboscia inermis, Thalassiosira tumida and Stellarima microtrias after exposure to 12.13 ± 2.13 W.m-2 (60.5 ± 10.6 μE m-2 s-1) PAR, 1.19 ± 0.68 W.m-2 UV-A and various UV-B irradiances.

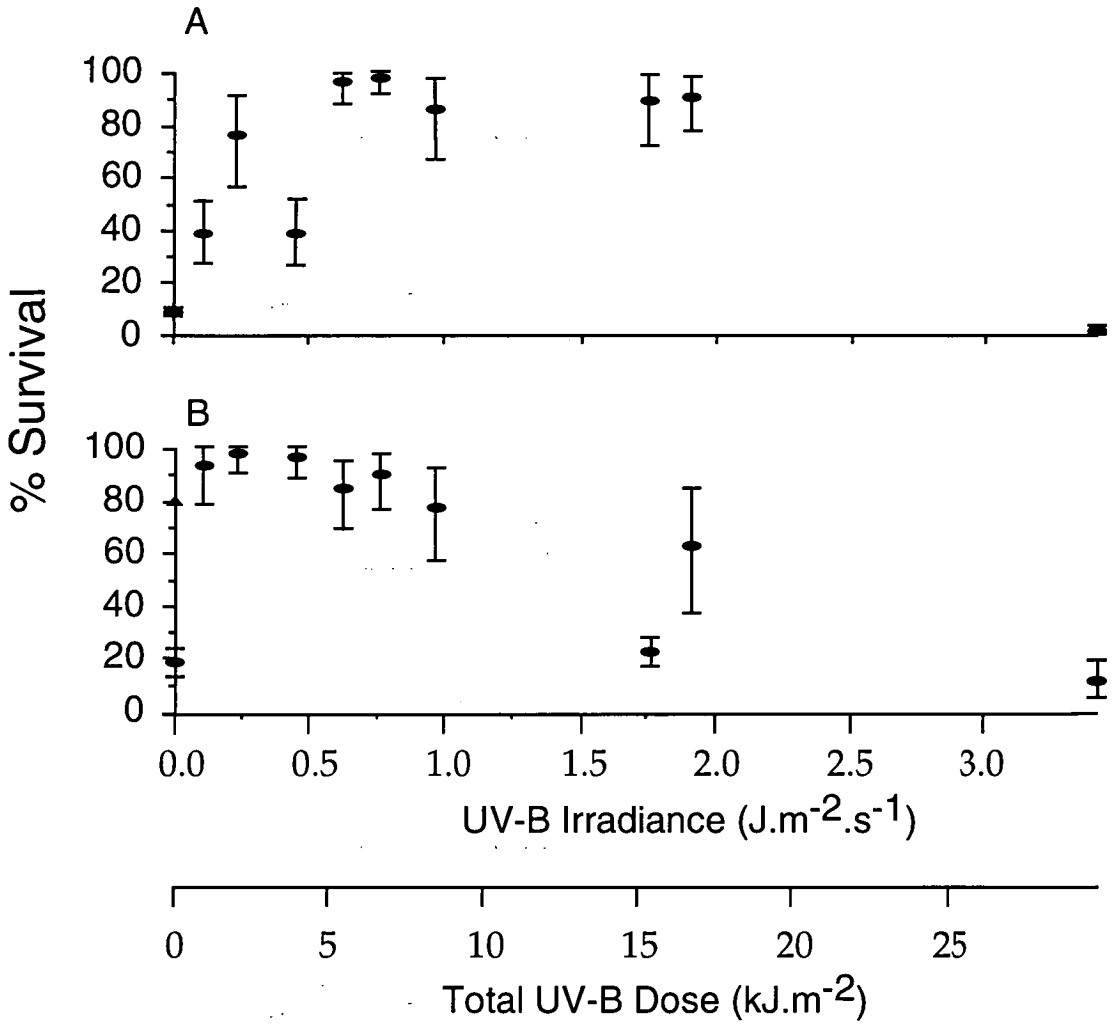


Fig. 7. Percent survival of (A) Nitzschia lecointei and (B) Stellarima microtrias after receiving 24 h continuous UV-B irradiation. Error bars represent standard errors calculated from Zar (1984).

over the lower range of UVB irradiances was approximately 20% lower in exposures with a dark period (Fig 5E) than those without (Fig. 7B).

4. DISCUSSION

This study was structured to approach natural conditions so that some insights into basic responses of diatoms to UVB exposure could be revealed. Experimental limitations included the inability to replicate the dynamic nature of the light climate of the Antarctic marine ecosystem in the laboratory and that the period over which the UVB irradiation treatments took place (48 hour period with 24 hour exposure to UVB) were relatively short . However, UVB exposure of 24 - 48 h reportedly produces significant changes in phytoplankton photosynthetic (Bidigare 1989) and UV–absorbing pigmentation (Marchant et al. 1991). Numerous Antarctic marine organisms have been shown to produce UV–absorbing compounds (Mitchell et al. 1989, Vernet et al. 1989, Karentz et al. 1991b, Marchant et al. 1991) and there is now a substantial literature indicating that such compounds, principally mycosporine-like amino acids (MAAs), can provide protection against UVB damage (eg Vernet et al. 1989, Carreto et al. 1990, Karentz et al. 1991b). Marchant et al. (1991) reported a high concentration of UV–absorbing compounds in the colonial stage of the Antarctic prymnesiophyte Phaeocystis antarctica and demonstrated that the colonial stage of this alga survived higher levels of UVB irradiation than either the motile stage, or the colonial stage of temperate strains of this alga which lacked or contained much lower concentrations of these compounds.

Only compounds with absorption at wavelengths greater than 290 nm were considered in this study as shorter wavelengths are not encountered in the marine environment (Smith & Baker 1979, Baker et al. 1980) and would be of no ecological significance in UV protection. The diatoms examined in this chapter have compounds that absorb at UVA wavelengths with only low absorbance by the shoulders of these peaks at UVB wavelengths. The concentrations of UV–absorbing compounds in the diatoms were approximately two to five orders of magnitude less per unit cell C than in Phaeocystis

antarctica and, unlike P. antarctica , the concentration of UV–absorbing compounds did not increase significantly as irradiance increased. Therefore, it appears that these diatoms are not using pigments as protection from UVB to the same extent as Phaeocystis. It remains possible that absorbance at these wavelengths is an incidental consequence of possessing certain cell proteins or metabolites which constitute a target rather than a protective mechanism. Thus, the significance of UV–absorbing compounds in the diatoms as a screen remains uncertain but appears low.

Phaeocystis antarctica contained high concentrations of UV–absorbing compounds (Marchant & Davidson 1991). The reports of substantial levels of UV–absorbing compounds in mixed phytoplankton from Antarctic waters by Mitchell et al. (1989), Vernet et al. (1989), and Gieskes and Kraay (1990) do not conflict with my data. Their unidentified samples could have contained P. antarctica, an abundant component of the Antarctic phytoplankton community or other as yet unidentified species which also possess such UV–absorbing compounds. However, data presented here demonstrates that diatoms, which contain little if any of these compounds to shield them from UVB exposure, are capable of surviving higher levels of UVB exposure than P. antarctica. Smith et al. (1992) also found that the rate of cell division of another diatom, Chaetoceros socialis, in the Southern Ocean was less affected by a given solar irradiance (including UVB) than P. antarctica.

With the exception of Thalassiosira tumida, absorption of UVB by cell concentrates from which MeTHF soluble pigments had been extracted was considerably greater than the maximum UV absorption by MeTHF–soluble pigments. Absorption of UVB by the frustule was similar to or greater than absorption by the MeTHF soluble pigments. Most of the UVB absorbance in all species except T. tumida was due to oxidisable cell contents. The location of these absorbing compounds and structures such as membranes, proteins and carbohydrates within the cell in relation to UV targets within the cell would determine their value as an intracellular screen against UVB damage. The low absorption

by MeTHF soluble pigments supports the argument that they are not primarily UVB protective compounds. Further, the UVB irradiance at which each species showed a significant decrease in survival did not correlate with absorption by MeTHF soluble pigments, frustules or MeTHF insoluble cell contents. This suggests that none of these fractions provide significant protection from UVB radiation and that processes other than UVB screening are responsible for survival of diatoms at elevated UVB irradiances.

The pycnocline in the MIZ may be 20 m or less for periods of up to 6 days (Mitchell & Holm-Hansen 1991, Veth 1991). Thus, phytoplankton in this environment may receive high UVB irradiances for prolonged periods. These results indicate that diatoms and Phaeocystis antarctica are able to survive and grow at UVB irradiances approximately twice, and in the case of Proboscia inermis and Stellarima microtrias, over three times the peak surface irradiance currently experienced in Antarctic waters for at least 24 hours. Their capacity to withstand UV exposure may reflect changes in species composition or selection of UV resistant strains over the 20 years of known existence of ozone depletion. Alternatively, high UVB environments may have existed for substantial periods in their evolution, thus pre-adapting these organisms (Yentsch & Yentsch 1982). Smith et al. (1992) found that the growth rates of phytoplankton after irradiation were independent of the depth from which the samples had been taken and depended only on the dose received at the depth of incubation. This evidence lends further weight to the idea that these organisms are pre-adapted to a relatively high UVB environment. These results also suggests that the impact upon diatoms of increased UVB irradiance as a result of ozone depletion may be minimal.

The survival of Thalassiosira tumida and Stellarima microtrias was less than 100% at UVB irradiances between 0 and 1.75 $J.m^{-2}$ s^{-1}. However, the survival at these irradiances did not appear to correlate with UVB irradiance and S. microtrias did not show any significant decline in survival until the highest UVB irradiance. Thus, the lower maximum survival probably reflects subculturing disturbance or overestimation of

the Time 0 population of the PAR-irradiated control culture. The low survival observed in all species under Mylar screens, which received no UVB but unattenuated UVA, indicates that UVA is also potentially lethal to phytoplankton but this damage is ameliorated by UVB. While photorepair of UVB-induced damage has been reported (Harm 1980, Karentz 1988, Karentz et al. 1991a), UVB-facilitated repair of UVA-induced damage has not. UVA is largely responsible for the inhibition of carbon fixation (Bühlmann et al. 1987) but it would appear unlikely that photoinhibition alone could be responsible for the observed mortality in Mylar screened treatments. The short wavelength UVA emitted from the UV lamps used in these experiments may have been affecting other cellular processes or constituents.

Ongrowth of cultures irradiated over 24 to 48 h indicated that the growth rate of most species was unaffected by the UVB irradiance to which they were subjected. This was observed even at irradiances which resulted in high mortality. Results indicate that cells which survived, sustained their metabolism during irradiation and were then able to resume normal growth, making their growth rate indistinguishable from both control cultures and those which received much lower UVB irradiances. Thus, their contribution to the population after irradiation is dependent upon their survival rather than any persisting consequence of the UVB dose received.

Estimates of mixing times from the surface layer to a depth of 10 m range from 30 minutes to hundreds of hours (Denman & Gargett 1983, Karentz 1991). Because of the stability of the MIZ, phytoplankton experience little or no darkness during late spring and summer (Sakshaug & Skjoldal 1989, Lizotte & Sullivan 1991, Veth 1991). Phytoplankton above the pycnocline will be exposed to changes in UVB irradiances over periods greater those used in the experiments (24 h). Therefore, these experimental results can only be considered as indicative of natural conditions. The two diatom species that received continuous irradiation at various UVB irradiances for a 24 hour period show a high tolerance to such an exposure. Dark–dependent DNA repair processes have been

cited as fundamentally important to many organisms (prokaryotes, plants and animals) for repair of UVB induced damage (Harm 1980). Over the 24 h duration of this experiment they appear to play little part in the survival and reproduction of Nitzschia lecointei and Stellarima microtrias denied a dark period.

Karentz et al. (1991a) found that smaller cells with a greater surface area : volume ratio were more sensitive to UV than larger cells. Results presented here are not consistent with this proposal. Survival of smaller species such as Nitzschia lecointei with a surface area to volume ratio approximating 0.94 was equivalent to that observed for Proboscia inermis with a surface area to volume ratio of around 0.20. The observations of Karentz et al. (1991a) may be due to cell size and volume affecting sinking rates, which in turn affects their relative exposure to UVB (Denman & Gargett 1983, Karentz 1991, Veth 1991, Thompson et al. 1991).

5. CONCLUSION

The results show that over a 24 to 48 hour period at least some diatoms tolerate levels of UVB considerably higher than irradiances received in Antarctic surface waters in the austral spring 1989. The concentration of UV–absorbing compounds in the diatom species investigated was much less than that observed in the haptophyte Phaeocystis antarctica, but their UVB survival tolerance exceeds that of P. antarctica. Thus, it cannot be assumed that survival of phytoplankton in high UV environments will be greater for species possessing UV–absorbing compounds. The high tolerance of UVB radiation of the phytoplankton species that were studied suggests that major changes in phytoplankton species composition as a result of extensive UVB induced mortality appears unlikely. Similarly, there is little direct evidence of changes in species composition in the Southern Ocean.

Photobiological responses to UV radiation are strongly influenced by dose rate and wavelength structure (eg. Smith & Baker 1979, Karentz 1991, Vincent & Roy 1993).

Laboratory experiments presented in Chapters 5 and 6 simulated or contained exposure times, wavelength structures and absolute irradiances that could be experienced in Antarctic waters. However, the inhibitory effect of UV radiation is a function of both UV dose and dose rate (Cullen & Lesser 1991) and such laboratory experiments cannot recreate natural diurnal and seasonal variations in irradiance and wavelength structure that result from physical and biological factors such as ozone concentration, solar elevation, cloud, ice cover and self-shading (Hardy & Gucinski 1989, Smith & Baker 1989, El-Sayed et al. 1990, Karentz 1991, Gautier et al. 1994). Chapters 7 and 8 report the results of experiments conducted at Davis Station, Antarctica, to examine the photobiological responses of Phaeocystis antarctica and selected species of Antarctic marine diatoms to natural or in situ Antarctic UV radiation.

CHAPTER 7

Comparative impact of in situ UV exposure on productivity, growth and survival of Antarctic Phaeocystis and diatoms

1. INTRODUCTION

Many authors have shown that UVB radiation (280 - 320 nm) reduces the survival, growth, and production of phytoplankton (See Chapter 1 section 6.1.1 & 6.1.2). Calkins and Thordardottir (1980) suggested that temperate and sub-polar diatoms possess little reserve capacity to cope with increased UVB exposure. El-Sayed et al. (1990) concluded that Antarctic phytoplankton are currently UV stressed and are likely to be seriously affected by any increase in UVB radiation. In contrast, studies of North American phytoplankton by Gala and Giesy (1991) and Hobson and Hartley (1983) found little inhibition of production by UVB and, in Antarctic waters, Helbling et al. (1994) calculated a negligible loss of phytoplankton production as a result of increased UVB. Davidson et al. (1994) found that selected species of Antarctic diatoms, though variable in their response, sustained no significant mortality until UVB exposures were increased to levels 2 to 3 times greater than those currently experienced in Antarctic surface waters. While the prospects for diatoms under increasing UVB irradiances are uncertain, tolerance of nanoplankton to UVB exposure is little known but apparently low (El-Sayed et al. 1990, Karentz et al. 1991a).

Marine phytoplankton productivity in surface waters is reduced under ambient and elevated levels of UV (eg. Worrest et al. 1981b, Bühlmann et al. 1987, El-Sayed et al. 1990, Smith et al. 1992, Behrenfeld et al. 1993, Prézelin et al. 1993, Helbling et al. 1994, Neale et al. 1994,). However, UVA wavelengths are not enhanced by ozone depletion and they have been found to be a major factor in depressing rates of

photosynthesis and growth (eg. Jitts et al. 1976, Jokiel & York 1984, Maske 1984, Bühlmann et al. 1987, Holm-Hansen et al. 1989, Helbling et al. 1992, 1994). Holm-Hansen et al. (1989) found that in near-surface Antarctic waters approximately 50% of inhibition of photosynthesis was due to UVA. UVB in near-surface waters may inhibit photosynthesis by between 15 and 60% (Worrest 1983, Maske 1984, Holm-Hansen et al. 1989, Helbling et al. 1992, Smith et al. 1992). However, the greater penetration of the water column by UVA than UVB meant that UVA was responsible for most of the photoinhibition in these waters (Holm-Hansen 1990). Long-term exposures of phytoplankton have also shown UVA is responsible for almost all inhibition of phytoplankton growth (Jokiel & York 1984).

While laboratory studies are necessary and important in revealing UVB tolerance mechanisms, ecologically relevant data can only be gathered using natural solar irradiances on natural phytoplankton populations. However, it is difficult to discern subtle phytoplankton responses to UVB exposure amongst the spatial and temporal variability of natural phytoplankton communities (eg. Vincent & Roy 1993). This chapter examines the in situ primary production, growth and survival of cultured Antarctic isolates of P. antarctica and selected species of diatoms at an Antarctic coastal site and their growth rate after irradiation.

2. MATERIALS AND METHODS

Unialgal cultures of Chaetoceros simplex, Stellarima microtrias, Fragilariopsis curta and Phaeocystis antarctica were isolated from Prydz Bay, Antarctica in 1991/92 and were maintained in culture under cool white fluorescent light at photosynthetically active radiation (PAR) intensity of 5.11 $W.m^{-2}$. C. simplex, S. microtrias and F. curta were grown in f/2 medium (Guillard & Ryther 1962) and a mixed flagellate and colonial life stage culture of P. antarctica was grown in GP5 (Loeblich & Smith 1968). An exponential-growth-phase culture of each species was diluted 1:6 with fresh nutrient medium two days before in situ incubation. Immediately before irradiation the cultures

were thoroughly mixed and 250 ml of each species transferred to each of three Whirlpak bags which transmitted light above 220 nm (PAR, UVA and UVB treatment). One bag remained unscreened while the remainder were screened with mylar (which transmitted wavelengths above 320 nm - PAR and UVA treatment) or polycarbonate (which transmitted wavelengths above 370 nm - PAR treatment). Like Prézelin and Smith (1993), no evidence of inhibition of growth or photosynthesis by UVB induced toxicity of Whirlpaks was found (Holm-Hansen & Helbling 1993). Interspecific differences in growth and photosynthesis were species specific rather than treatment dependant. Bags were then incubated at 0.30 m depth in near-shore waters off Davis between 19th Feb and 26th Feb 1992.

A further seven 50 ml subsamples of each species were transferred to 100 ml Whirlpak bags for primary production incubations. Three bags were screened as above, one was screened with opaque black plastic as a dark bag control and a further three were immediately acidified with 200 µl of 6 N HCl as time zero blanks. Primary production was estimated using the methods of Schindler et al. (1972) modified after Griffith (pers. comm.). At the conclusion of the production incubation a 7 ml subsample from each Whirlpak was transferred to a 20 ml scintillation vial and acidified as above. The vials were then shaken at 200 rpm for 2 hours to remove inorganic ^{14}C. Counts were performed in Lumagel using a LKB 1215 Rackbeta II liquid scintillation counter. Estimates of count efficiency were performed each sample day before performing decay counts. The mean of triplicate time zero blanks and dark bag uptake were subtracted from counts in calculation of primary production. In situ incubations were performed at 0.30 m depth for 4 hours between 10.30 and 12.30 solar time. Determination of primary production by each species and under each light treatment were repeated after 4 and 8 days of in situ incubation. The light treatment of each primary production incubation was the same as that from which the subsample was removed.

Surface UVA and erythemal UVB irradiance was integrated in situ using an International Light IL 1700 Radiometer. Primary calibration of detector response was made using a National Institute of Standards and Technology intercomparison package (NIST Test # 534/240436-88) with further calibration using four International Light primary transfer standards. A secondary calibration of the sensors to solar irradiances was conducted using the sensor response curve and a Macam spectroradiometer and erythemal UVB biometer respectively.

A 5 ml subsample of each in situ incubated treatment for each species was inoculated into 30 ml of fresh growth medium. These cultures were returned to culture maintenance conditions for estimation of growth rate and survival and will henceforth be referred to as "ongrowth" cultures. A further 10 ml was removed at each sample time and fixed with Lugol's iodine for estimation of cell concentration using inverted microscope cell counts over 15 replicate fields. Cell concentration in ongrown cultures was estimated 3 and 9 days after subculturing and the growth rate of the control culture then used to calculate the number of surviving cells immediately after irradiation from the final cell concentration in irradiated treatments (Davidson et al. 1994). Calculations ensure that only viable cells capable of contributing to population growth are included in the survival of each species under each light treatment. After 2, 4, and 8 days in situ exposure subsamples were removed from each 250 ml Whirlpak and the in situ cell concentration, survival and rate of ongrowth again estimated.

The equivalent spherical diameters of P. antarctica flagellate and colonial cells were measured microscopically using a Zeiss Photomicroscope II at 1000 x magnification. A total of 200 equivalent spherical diameters were measured from each light treatment which had been irradiated for 8 days and ongrown for a further 9 days.

3. RESULTS

Surface UVA and UVB irradiances were integrated during the duration of the 8 days in situ incubation (Fig. 1, 3 & 4) and during each 4 hour primary production incubation (Fig. 5 & 6). Surface irradiances were high during the first 2 days of incubation as were irradiances during the primary production incubations. Between days 2 and 4 conditions were frequently overcast and surface irradiances were low, particularly at UVB wavelengths. Irradiances during primary production incubation were similarly low. Between day 4 and 8 integrated UVA and UVB irradiance increased again and surface irradiances integrated over the duration of the primary production incubation were the highest observed.

The concentration of colonial Phaeocystis antarctica changed little during in situ incubations (Fig. 1A). Samples which received UVB in the irradiance did not differ significantly from those that received PAR and UVA. Only in the incubation which received PAR alone may cell concentration have increased but this never differed significantly from UV exposed treatments. Exposure of colonial P. antarctica to unscreened solar irradiance (PAR, UVA and UVB) for periods of more than 2 days greatly increased their rate of post-irradiance ongrowth (Fig. 1B). Colonial cells which received PAR and UVA also showed a marked but lesser promotion of growth rate while growth of PAR irradiated control samples showed little increase in growth rate with incubation time.

The concentration of flagellate cells fell as a result of in situ UV radiation. Flagellate concentrations in the PAR irradiated treatment remained approximately constant (Fig. 1C). Cells subject to PAR and UVA declined to around 20% of their original numbers over the 8 day period while flagellate concentrations exposed to PAR, UVA and UVB declined at a similar rate but where almost absent after 8 days incubation. The rate of ongrowth of the flagellate stage after irradiation changed little with time irrespective of

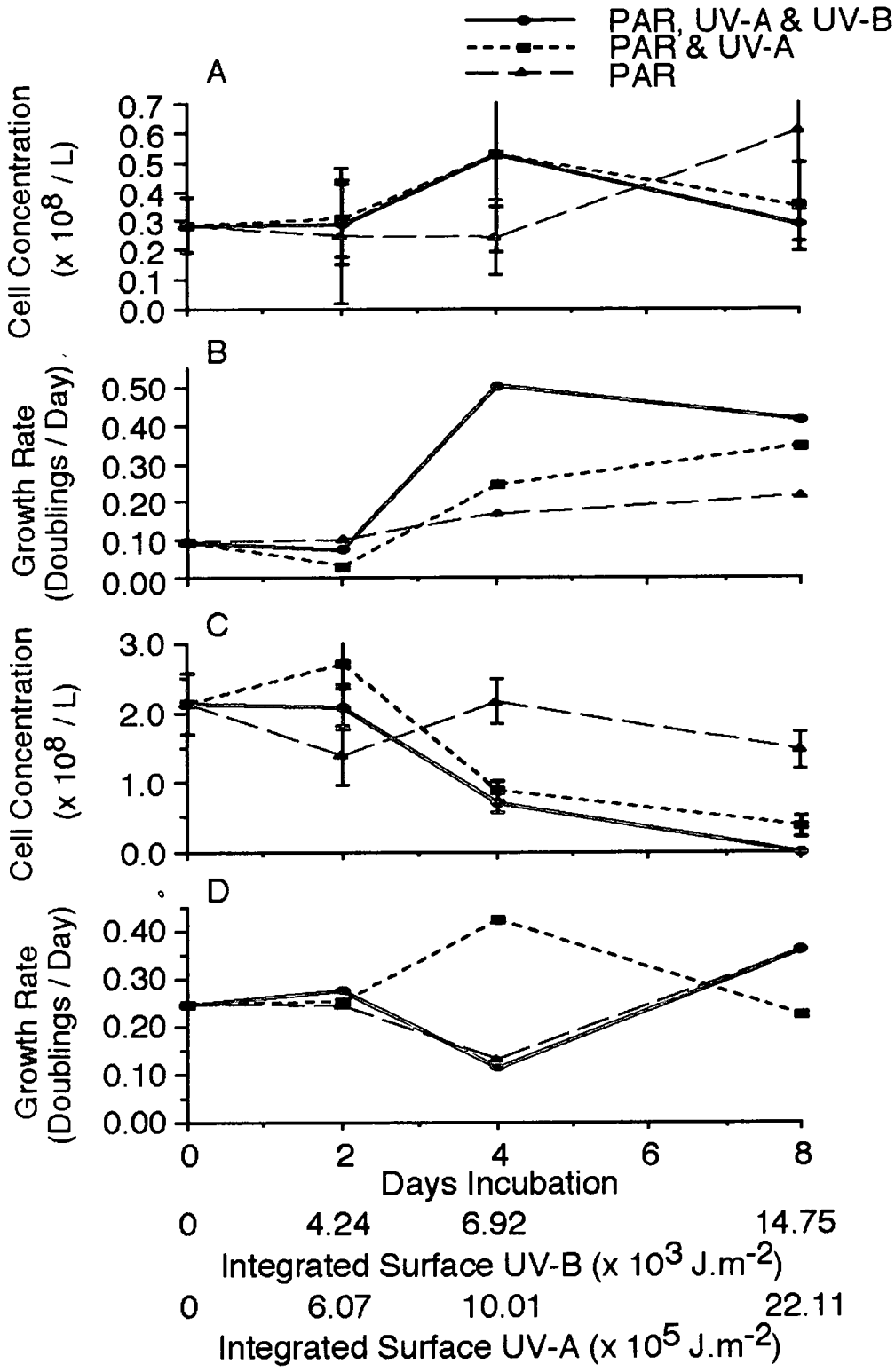


Fig. 1. Colonial Phaeocystis antarctica (A) cell concentration during in situ irradiation and (B) growth rate of cells incubated for 0, 2, 4 and 8 days, subcultured, returned to culture maintenance conditions and ongrown for 9 days. Flagellate stage P. antarctica (C) cell concentration during in situ irradiation and (D) growth rate after irradiation (as above). Growth rate calculated after Verity et al. (1988). Total integrated UVA and UVB dose at each in situ sample period are given. Error bars represent standard deviation.

irradiance treatment (Fig. 1D). The only exception was the PAR and UVA treatment after 4 days incubation, the reasons for which are uncertain.

The cell diameter of the colonial and flagellate cells increased with addition of UVA and UVB to the irradiance (Fig. 2A & B). Mean flagellate cell diameter in cultures receiving PAR were 3.18 μm (Fig. 2A). This increased to 3.71 μm with addition of UVA to the exposure and reached 4.50 μm when also exposed to UVB. The mean cell diameter of the colonial stage was 5.03 μm after exposure to PAR only (Fig. 2B). This increased to 6.18 μm with the introduction of UVA and further increased to 6.59 μm after exposure to UVA and UVB.

Exposure of colonial stage Phaeocystis antarctica to natural irradiances over a period of 8 days caused no decline in survival (Fig. 3). Survival of flagellate stage P. antarctica also remained high for the first 4 days incubation but declined markedly between days 4 and 8. The decline was greatest when cultures were exposed to the PAR, UVA and UVB but a major decline was also observed in the treatment with PAR and UVA.

The concentration of Chaetoceros simplex and Stellarima microtrias cells did not increase significantly during in situ incubation (Fig. 4A & B). Concentrations of Fragilariopsis curta did significantly increase in all treatments. The greatest increase was observed in the unscreened treatment during the first 4 days of irradiation after which the concentration declined toward day 8 (Fig. 4C). None of the diatom species exhibited any significant decline in the survival as a result of UV irradiance (Table 1). Interspecific differences were observed in the growth rate of cultures established and ongrown after irradiance treatments (Table 2). Growth of S. microtrias and C. simplex declined with the addition of UVA and UVB to the irradiance. UVB was responsible for the greatest decline in the growth rate of S. microtrias while the greatest decline in growth rate of C. simplex was caused by UVA. Fragilariopsis curta showed a promotion of growth rate in the unscreened treatment similar to that observed for P. antarctica. Unlike P. antarctica, little promotion of growth rate resulted from addition of UVA to the irradiance.

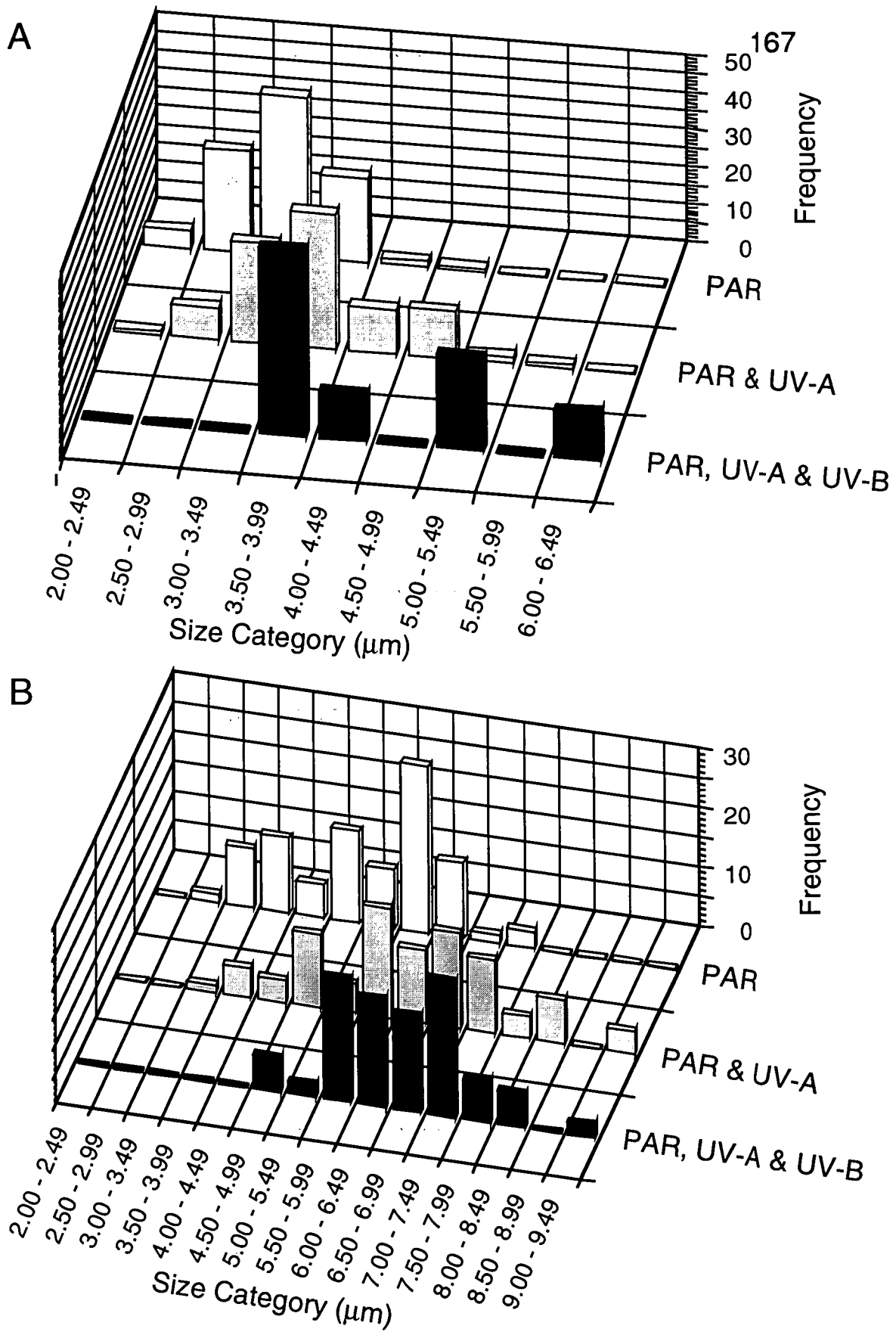


Fig. 2. Cell size distribution of (A) flagellate and (B) colonial cells Phaeocystis antarctica incubated in situ at 0.30 m depth for 8 days exposed to PAR, PAR and UVA or PAR, UVA and UVB subcultured and ongrown in culture maintenance conditions for 9 days.

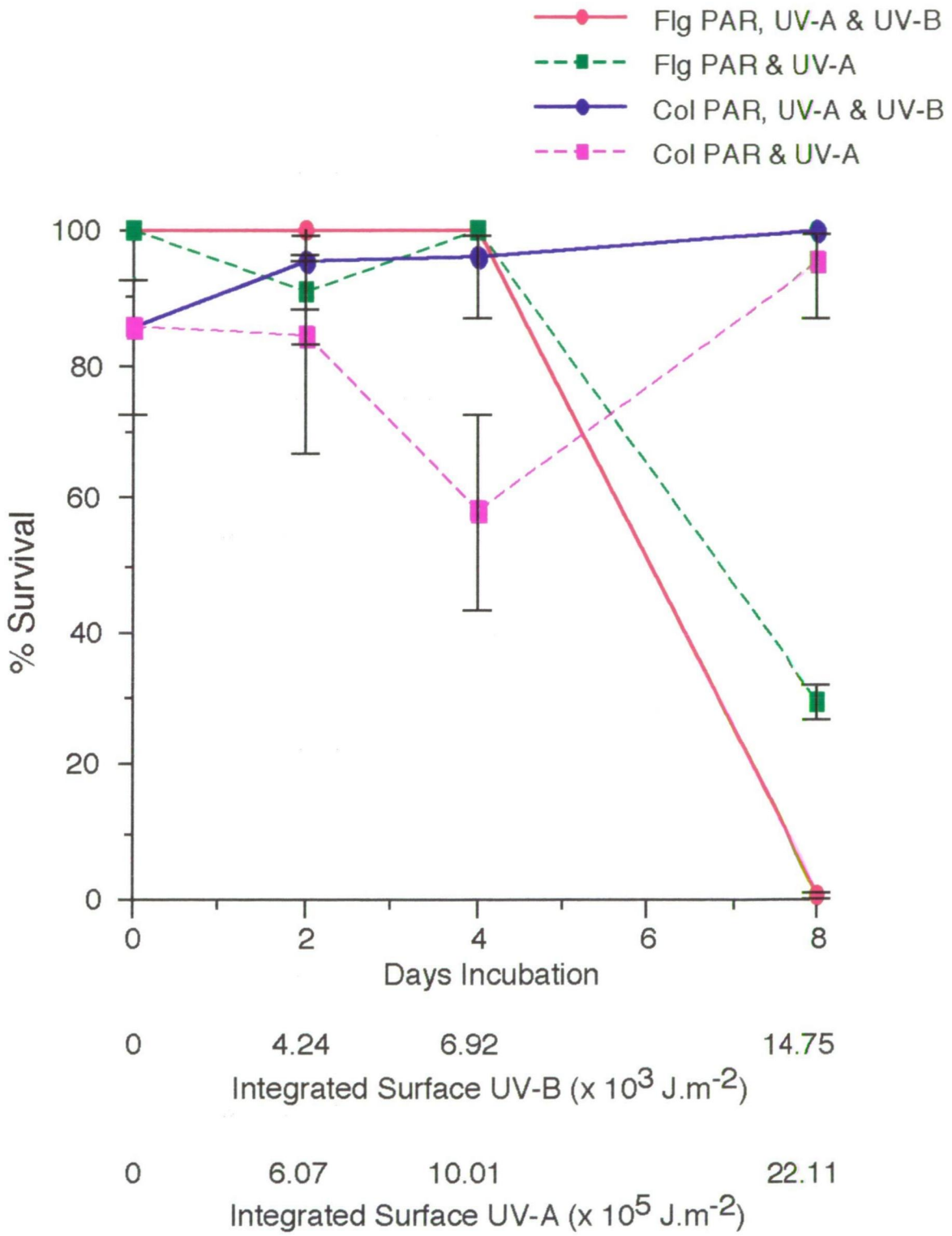


Fig. 3. Percent survival of unscreened (Flg PAR, UVA & UVB) or mylar screened (Flg PAR & UVA) flagellate stage and unscreened (Col PAR, UVA & UVB) or mylar screened (Col PAR & UVA) colonial stage Phaeocystis antarctica culture during near surface in situ incubations. Error bars represent standard error calculated after Zar (1984).

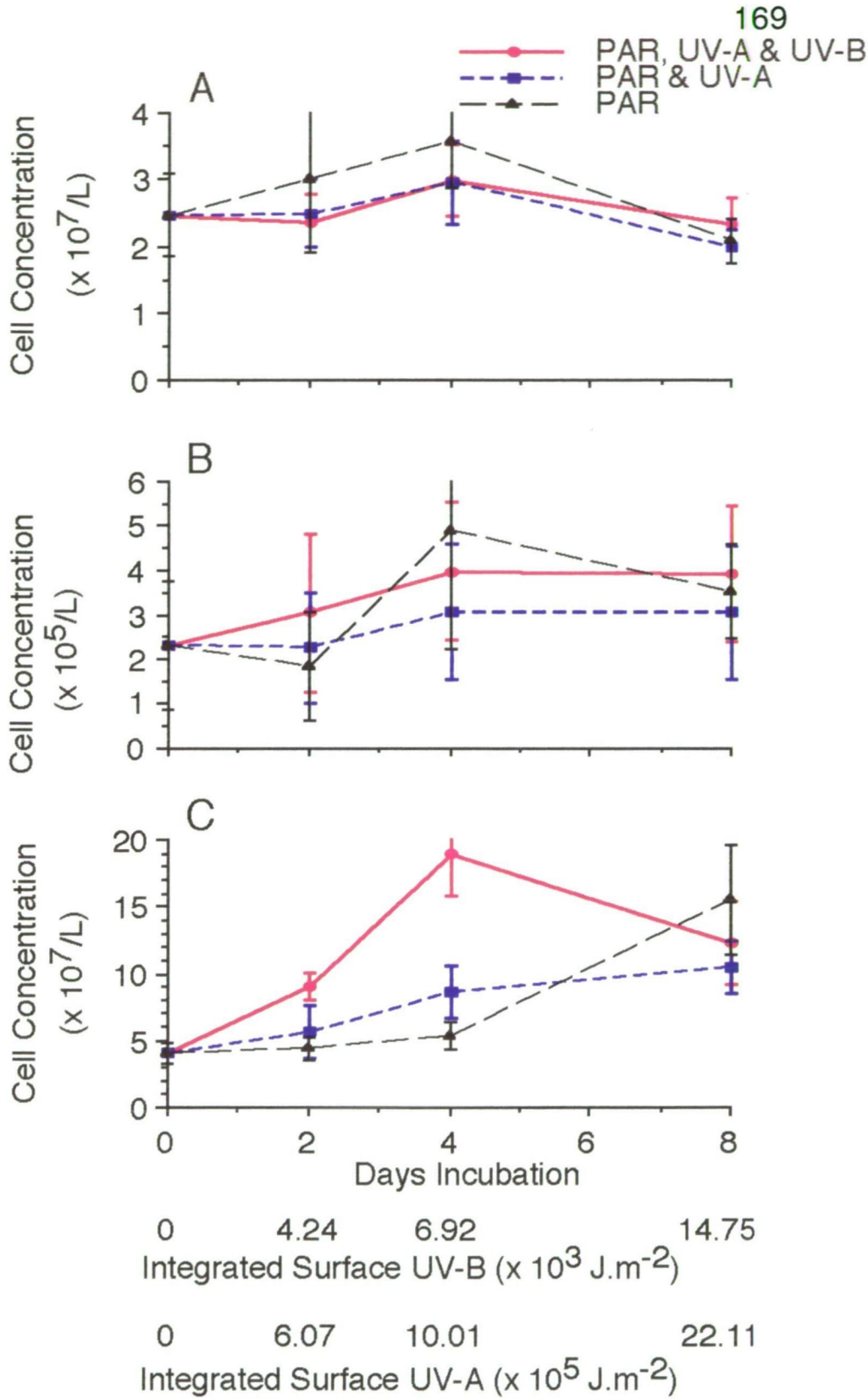


Fig. 4. Cell concentration of (A) Chaetoceros simplex, (B) Stellarima microtrias and (C) Fragilariopsis curta exposed to PAR, PAR and UVA or PAR, UVA & UVB during near surface in situ incubations. Total integrated UVA and UVB dose at each in situ sample period are given. Error bars represent standard deviation.

Table 1. Percent survival of PAR and UVA or PAR, UVA and UVB irradiated diatoms exposed to near surface in situ irradiance for 8 days calculated after Davidson et al. (1994). L1 and L2 represent upper and lower standard errors calculated after Zar (1984)

Species	PAR & UVA			PAR, UVA & UVB		
	Mean	L1	L2	Mean	L1	L2
S. microtrias	95.88	99.87	86.77	93.53	98.55	85.28
C. simplex	99.17	99.97	96.04	98.59	99.91	95.70
F. curta	100.00	100.00	100.00	100.00	100.00	100.00

Total photosynthetic rates of Phaeocystis antarctica only declined slightly with incubation time and little difference was observed between the irradiance treatments (Fig. 5A). The carbon fixation rate per cell in the PAR screened treatment also exhibited little change with time (Fig. 5B), however, fixation rates per cell in treatments which receiving UVA or UVA and UVB increased rapidly. This resulted from the decrease in flagellate cell concentration (Fig. 1C). In addition, the irradiance treatment and the flux rate during the production incubation appear to have little effect on the rate of production by the colonial stage (Fig. 5A). The diatom species investigated showed differing responses in production to the irradiance treatment. Although rates of production were frequently lowest in treatments which received UVB, inhibition of photosynthesis was only slight. The rate of production per cell by the diatom species investigated was not reflected in changes in cell concentration during in situ incubation. Little difference was observed in primary production per cell of Chaetoceros simplex between light treatments, however, the production by each cell approximately doubled during in situ incubation (Fig. 6A). Production per cell by Stellarima microtrias appeared to decline slightly during incubation

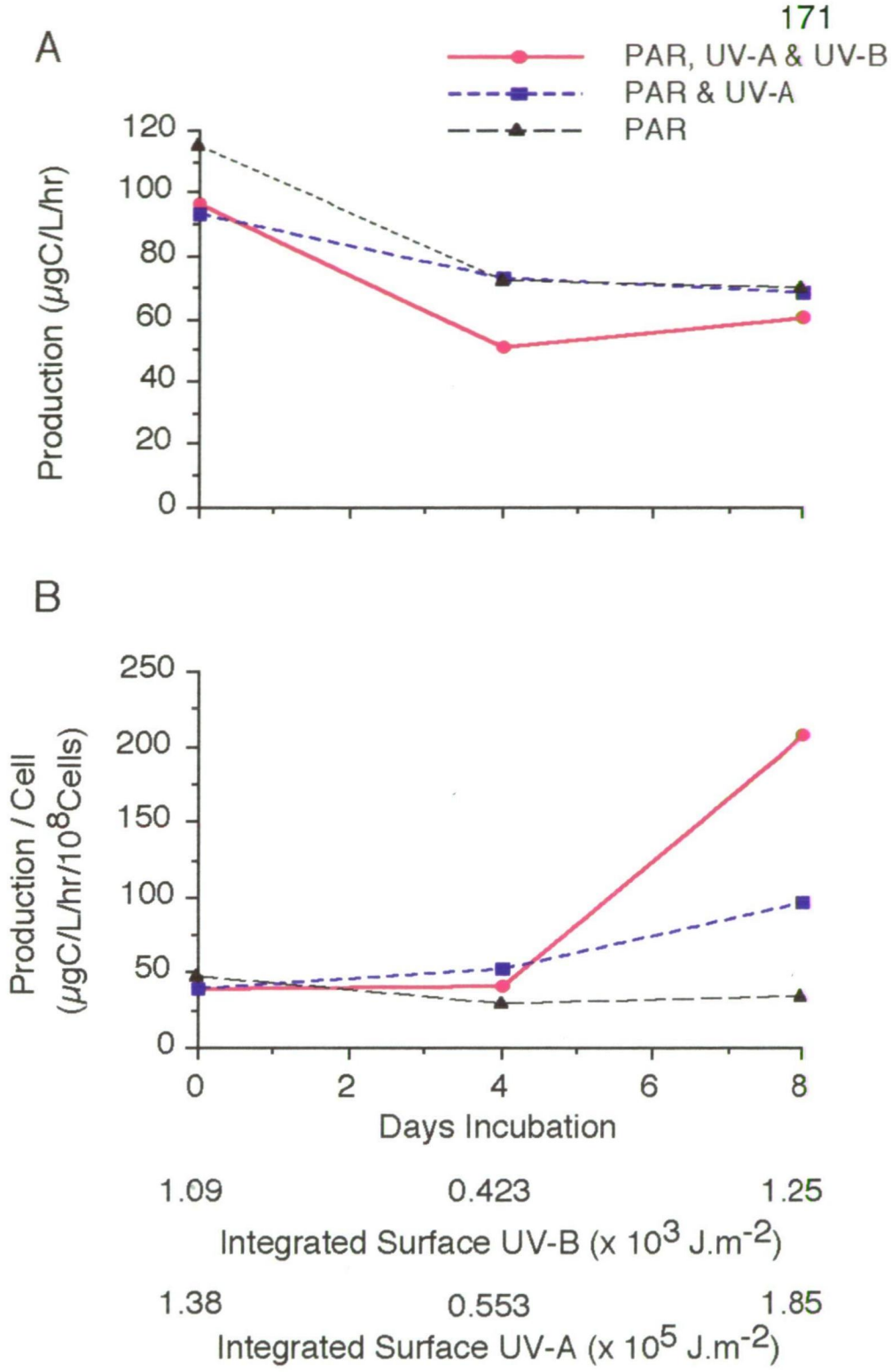


Fig. 5. The rate of (A) primary production and (B) production per cell by cultured Phaeocystis antarctica taken from near surface in situ incubations. 50 ml sub-samples were removed from polycarbonate, mylar or unscreened light treatments and replaced in situ beneath the same screen for 4 hr incubations to estimate primary production. Surface UVA and UVB irradiance was integrated for the duration of the production incubations

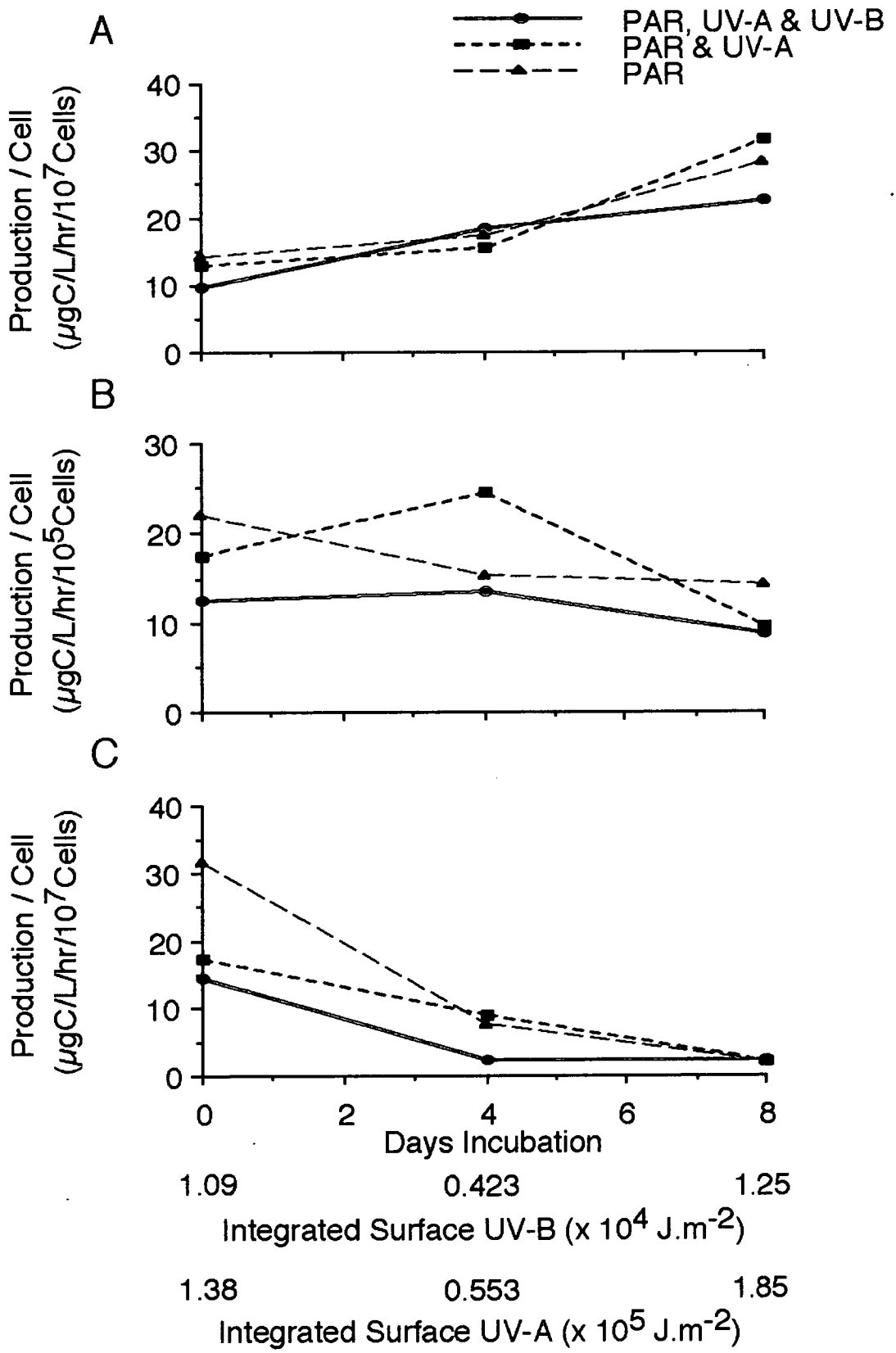


Fig. 6. The rate of primary production per cell by (A) Chaetoceros simplex, (B) Stellarima microtrias and (C) Fragilariopsis curta during near surface in situ incubations performed as for Phaeocystis antarctica.

(Fig. 6B), while that by Fragilariopsis curta declined by approximately 90% in all treatments (Fig. 6C).

Table 2. Growth rate of diatoms after 8 days near surface in situ exposure to PAR, PAR and UVA or PAR, UVA and UVB, subcultured and ongrown in culture maintenance conditions for 9 days. Growth rate calculated after Verity et al. (1988a)

Species	Growth Rate PAR	PAR & UVA	PAR, UVA & UVB
S. microtrias	0.263	0.218	0.122
C. simplex	0.674	0.336	0.266
F. curta	0.253	0.289	0.657

4. DISCUSSION

4.1. Survival

Flagellate stage Phaeocystis antarctica was the only organism examined which demonstrated a significant decline in cell concentration during in situ exposure and survival after irradiation. UVA was responsible for most of this decline. Jokiel and York (1984) found that long term inhibition of growth was due almost entirely to UVA. Results presented here indicate that it can also account for most of the mortality. Addition of UVB to the irradiance further reduced the cell concentration of the flagellate stage but differences were slight and only significant after 8 days irradiation. Karentz (1991) and Calkins and Thordardottir (1980), indicate that UVB induced mortality would act as a selective pressure on the species composition of the phytoplankton community. On the basis of monospecific culture growth, and since UVA irradiances are not significantly enhanced as a result of ozone depletion, the results presented indicate that no major decline in P. antarctica or diatom abundance as a result of UVB induced mortality is likely.

The colonial stage of Phaeocystis antarctica possesses high concentrations of UV-absorbing compounds which provided substantial protection from UV radiation (Marchant et al. 1991). The flagellate stage lacks these compounds and exhibited greater vulnerability to UV radiation. The in situ results presented here support the previous finding. Antarctic near surface UV irradiances are sufficient to cause mortality in flagellate P. antarctica populations but the colonial stage maintains its photosynthetic production during exposure to UV and exhibits increased growth and cell size, and high survival after irradiation with UVA and UVB.

4.2. Growth

UV is reportedly responsible for significant decreases in the growth rate of phytoplankton (Thomson et al. 1980, Hannan et al. 1980, Worrest et al. 1981b, Jokiel & York 1984, Döhler 1984, 1985, Karentz et al. 1991a, Smith et al. 1992, Holm-Hansen et al. 1993, Villafañe et al. 1995). Worrest et al. (1981b) attributed this to interspecific differences in genetic limits of photoadaptation. In situ incubation results presented in this chapter, using diluted exponential growth phase cultures, found only Fragilariopsis curta sustained significant growth. This suggests that growth may have been inhibited as a result of PAR, however, Jokiel and York (1984) found high levels of PAR were not inhibitory to growth. Alternatively, features of the in situ environment such as temperature may have reduced growth rates. Smith et al. (1992) found that growth of P. antarctica (presumably colonial) in Antarctic waters was inhibited by inclusion of UVB in the natural solar irradiance while the growth of Chaetoceros socialis was not. No significant inhibition of colonial P. antarctica growth by UVB was observed in this study but this may have been due to the use of monospecific nutrient enriched cultures (Döhler 1992, Lesser et al. 1994) or differences in experimental methods and strain.

In situ exposure of Phaeocystis antarctica to UV resulted in an increase in cell diameter in both the flagellate and colonial life stages of subcultures established immediately after irradiation and allowed to grow in culture maintenance conditions for a further 9 days. An

increase in cell size may be caused by inhibition of cell division (Badour 1968) or an increase in light intensity (Thomson et al. 1991). The concentration of flagellate cells decreased in irradiances treatments including UVA or UVA and B but their growth rate after irradiation did not differ markedly between irradiance treatments, indicating that flagellate cells which survived irradiation were able to sustain normal growth. No significant change in colonial cell concentration was observed in any of the irradiance treatments during in situ irradiation and those that received UVA or UVA and UVB exhibited enhanced growth rates after irradiation. Thus, unlike Badour (1968), the observed increase in cell size as a result of exposure to UV radiation appear not to be as a result of inhibition of cell division. The increase in cell size observed by Thomson et al. (1991) were reversible after 12 hours while the increase presented here is wavelength dependant and persisted for at least the 9 days of ongrowth. Results presented in this chapter indicate a sustained change in cell metabolism of UV irradiated P. antarctica. Changes in size of the flagellate cells may, however, also reflect UV induced changes in flagellate cell stage (Kornmann 1955, Paperzak L 1992 pers. comm.) or formation of flagellates from the colonial stage (Veldhuis et al. 1986b, Verity et al. 1988a, Davidson & Marchant 1992a, b).

Exposure of Fragilariopsis curta to PAR, UVA and UVB caused a rapid increase in cell concentrations for the first 4 days of incubation suggesting high UV tolerance by this species. UV irradiances between days 4 and 8 were high. During this time the concentration of F. curta in this treatment declined suggesting that exposure of the cells beyond an upper threshold becomes inhibitory to their growth or that the UV exposure may impose cumulative stress on cell physiology which is expressed only after extended periods of irradiation (Calkins & Thordardottir 1980, Jokiel & York 1984, Döhler 1984, Vosjan et al. 1990, Marchant et al. 1991, Behrenfeld et al. 1992, Bothwell et al. 1993, Davidson et al. in press). However, Bothwell et al. (1993) and Villafañe et al. (1995) observed a reversal of inhibitory effects in long–term exposures to solar UV radiation (3 - 5 weeks).

Interspecific differences were observed in the growth rate of cultures established and ongrown after irradiance treatments. Ongrowth of Fragilariopsis curta showed the division rate of the PAR, UVA and UVB irradiated treatment for this species was more than twice that of other treatments despite its rates of primary production during incubation being low. To sustain growth after irradiation the photosynthetic rate of F. curta must recover rapidly, however, the differences in the rate of ongrowth by F. curta must largely reflect UVB induced effects on processes other than photosynthesis. Davidson et al. (1994) suggests the possibility of UVB being involved in repair of UVA related damage. This may explain the higher growth rate of the unscreened treatment than that receiving UVA. The reason for the lower growth rate of the PAR irradiated ongrowth culture is unclear but, like colonial Phaeocystis antarctica, exposure to UV may promote growth after irradiation. Ongrowth of Stellarima microtrias showed greatest reduction in growth rate as a result of UVB irradiance. That of Chaetoceros simplex was reduced most by UVA but declined further with addition of UVB to the irradiance. The reduced rate of ongrowth by these species may, at least in part, reflect the degree of inhibition of photosynthesis by UV during in situ incubation.

The photobiological strategy favoured as a result of UVB exposure would depend on the duration and intensity of the irradiance received. Though the diatoms examined here survive high UV irradiances for a short time (Davidson et al. 1994) their long term survival and growth during and after irradiation may not advantage them over species that appear more vulnerable. For example, Stellarima microtrias is able to survive UVB intensities approximately an order of magnitude higher than that of Phaeocystis antarctica (Davidson et al. 1994), however, it grows little better than P. antarctica during in situ incubation and irradiation with UVB results in reduced growth after exposure. The rate of ongrowth for P. antarctica after exposure to UVB irradiation was approximately 3 times that of the PAR irradiated culture and this species would likely be favoured at sublethal irradiances.

4.3. Production

UV is widely reported as being inhibitory to photosynthesis (eg. Jitts et al. 1976, Lorenzen 1979, Worrest et al. 1981b, Jokiel & York 1984, Worrest 1986, Smith & Baker 1989, Voytek 1989, Häder & Worrest 1991). Estimates of inhibition by near surface UVB irradiances range from 15 - 30% while UVA resulted in a further decline of around 50% (Maske 1984, Holm-Hansen et al. 1989, Holm-Hansen 1990, Helbling et al. 1992). In Antarctic waters, the increase in UVB as a result of ozone depletion apparently results in a reduction of at least 6 - 12 % in primary production (Smith et al. 1992). Results presented here found inhibition of production was variable, probably as a result of variations in tolerance and photoadaptive ability of each species and changes in the in situ irradiance received. Photoinhibition was frequently greatest for treatments which received UVB in the irradiance but differences between light treatments were slight and percent inhibition seldom reached the magnitude reported above. However, these experiments used nutrient enriched monospecific cultures. The lower sensitivity to UVB may reflect the high nutrient environment (Cullen & Lesser 1991, Döhler 1992)

The colonial cell concentration remained relatively constant during the 8 days of irradiation. Exposure of flagellate cells to UV for periods exceeding 2 days significantly reduced their concentration in culture. However, in comparison with the PAR irradiated control, the rate of production in UV irradiated treatments did not markedly decline and the production per Phaeocystis antarctica cell greatly increased. Although no size fractionated production was conducted to separate the flagellate and colonial stages of P. antarctica, the colonial stage in the life cycle of this alga appears largely responsibly for photosynthesis during in situ incubation. This may be as a result of possessing UV–absorbing compounds (Marchant et al. 1991) which protect the photosynthetic apparatus (Vernet et al. 1994) and/or the sustained changes in physiology as a result of UV exposure. Chaetoceros simplex was the only diatom which increased its rate of photosynthesis per cell during in situ incubation suggesting photoadaptation of this

species to the near surface light environment. Primary production by Fragilariopsis curta declined markedly but this was apparently largely due to PAR irradiance rather than UV wavelengths. This contrasts with the finding of previous authors that PAR has little inhibitory effect upon photosynthesis (Jokiel & York 1984, Bühlmann et al. 1987).

5. CONCLUSION

The nature and duration of UV exposure in Antarctic waters is yet to be fully determined. Shallow blooms of the MIZ, which are responsible for much of the primary production in the Southern Ocean, appear vulnerable to increased UVB radiation as a result of stratospheric ozone depletion (Marchant & Davidson 1991). Interspecific differences in the responses of the phytoplankton to UV exposure have led to the suggestion that species or strains possessing greater tolerance to UV will be favoured (Worrest et al. 1978, 1981a, b, Häder & Worrest 1991, Karentz 1991, Marchant & Davidson 1991). However, the results presented in this chapter indicate that the interaction of UV intensity, dose and the photobiology of each species is complex and the impact on the organisms is not great. The consequent changes in phytoplankton species composition may be sufficiently slow or slight that they are undiscernible from spatial and interannual variability.

Like previous authors (eg. Worrest et al. 1978, 1981a, b, Calkins & Thordardottir 1980, Ekelund 1990, Karentz et al. 1991a, Helbling et al. 1992, Bothwell et al. 1993, Villafañe et al. 1995), results presented here show interspecific differences in the response of phytoplankton to UVB exposure. These differences suggest, but do not directly demonstrate, that UVB will cause changes in species composition. Chapter 8 investigates the impact of natural Antarctic UVB on phytoplankton species composition.

CHAPTER 8

Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culture

1. INTRODUCTION

Interspecific variation in survival, growth and repair responses to UVB exposure is reportedly high (Calkins & Thordardottir 1980, Worrest et al. 1981b, Jokiel & York 1984, Karentz et al. 1991a, Smith et al. 1992), even within a single genus (Mitchell & Karentz 1990). This has lead to the proposal that increased UVB irradiance is likely to alter the species composition of phytoplankton communities in favour of those species with greater tolerance (Calkins & Thordardottir 1980, Worrest et al. 1978, 1981b, Worrest 1983, Jokiel & York 1984, El-Sayed et al. 1990, Karentz 1990, 1991, 1994, Häder & Worrest 1991, Karentz et al. 1991a, Marchant & Davidson 1991, Helbling et al. 1992, Smith et al. 1992, Vincent & Roy 1993, Davidson et al. 1994). Long term exposure of natural phytoplankton assemblages to in situ UV irradiances reportedly changes the community composition (Worrest et al. 1981a, b, Bothwell et al. 1993, Villafañe et al. 1995). Interspecific differences in the tolerance of Antarctic phytoplankton to UVB have been reported (Karentz et al. 1991a, Marchant et al. 1991, Smith et al. 1992, Davidson & Marchant 1994, Davidson et al. 1994). Although studies have demonstrated differing tolerance to UVB exposure, no direct evidence of changes in species composition has not been reported for the Southern Ocean.

Experiments examining UVB–induced changes in species composition have been conducted over long time periods (Worrest et al. 1978, Thomson et al. 1980, Worrest et al. 1981b, Behrenfeld et al. 1992, Bothwell et al. 1993, 1994, Villafañe et al. 1995). However, marine phytoplankton inhabit a mixed environment and cells would seldom, if

ever, be exposed to high UVB intensities for such long periods. The period of exposure, eliciting species specific differences in the rates of damage, repair, adaptation and tolerance to UVB exposure, are likely to be critical in determining the occurrence and magnitude of changes in species composition (Chapter 1 section 6.1.2). The mixed depth in the marginal ice zone (MIZ) may be 20 m or less for up to 6 days (Mitchell & Holm-Hansen 1991, Veth 1991). Results presented in this chapter examine changes in species composition during exposures over only a few days.

Species specific investigations of the tolerance of phytoplankton to UVB irradiance could be important in predicting the effect of ozone depletion. However, such studies do not include the competitive interactions between phytoplankton species during UVB exposure. This chapter reports changes in species composition in competition experiment containing the flagellate and colonial life stages of Phaeocystis antarctica and five species of commonly occurring Antarctic marine diatoms in mixed culture during exposure to natural and attenuated UV irradiance.

2. MATERIALS AND METHODS

Unialgal strains of the diatoms Chaetoceros simplex, Fragilariopsis lecointei, Fragilariopsis curta, Thalassiosira tumida, Proboscia (Rhizosolenia) alata and the haptophyte Phaeocystis antarctica were isolated from Prydz Bay, Antarctica. Cultures were maintained under cool white fluorescent light at a photosynthetically active radiation (PAR) intensity of 5.11 Wm^{-2} and at 0°C with an 18:6 h light:dark cycle. Exponentially growing cultures of the six species (that of P. antarctica containing approximately equal concentrations of the flagellate and colonial life stages) were diluted 1 : 5, culture : fresh culture medium, 5 and 2 days before starting the experiment. Organisms in 10 ml subsamples of each monospecific culture were fixed with buffered Lugol's solution and the cell concentration estimated using the Utermöhl sedimentation technique over 15 replicate randomly chosen fields using an inverted microscope. Aliquots of each culture were mixed to give approximately equal cell concentrations of each species and both P.

antarctica life stages. Three 10 ml subsamples were removed to determine cell concentrations (as above) of each species at time 0. Nine subsamples, each of 500 ml were then transferred to polythene bags (Whirlpak, Nasco) which transmit light above 220 nm . Three replicate bags were exposed to one of three light treatments; unscreened (PAR, UV-A and UV-B treatment), Mylar screened (which transmitted wavelengths above 320 nm - PAR + UV-A treatment), and polycarbonate screened (which transmitted wavelengths above 370 nm - PAR treatment) (Davidson & Marchant 1994).

Mixed phytoplankton populations were incubated for 8 days at Davis Station, Antarctica (68°35' S, 78° E) at a depth of 0.2 m between 10th and 18th December 1992 in an outdoor tank through which sea water was circulated. Thus, the phytoplankton were exposed to near-surface natural light irradiance. Integrated irradiances were measured using an IL 1700 research radiometer equipped with UVA and erythemal UVB sensors (Davidson & Marchant 1994, Chapter 5 Fig. 1C) which were calibrated to solar irradiances using the sensor response curve and a Macam spectroradiometer and erythemal UVB biometer respectively. Sensors were positioned beside phytoplankton at 0.2 m depth and the UVA and UVB irradiance integrated during incubation.

A subsample of 10 ml was removed from each replicate treatment at two day intervals for 8 days and the concentration of each species determined (as above). The cell concentrations of each species after each period of irradiation, were used to estimate a single exponential growth rate for each replicate. This provided independent estimates of growth rate, with estimated variances for each species under each of the three UV treatments. Exponential ($\log_e$) growth rate estimates were obtained as the slope parameters of a generalised linear model (GLM) (see Chambers & Hastie 1993), using S-Plus statistical package with gaussian errors, a log-link function, and weighted by the inverse of the square of the empirical standard error for each cell concentration determination.

The growth rate of cells of each species in the culture became the dependent variable in a fully crossed two way analysis of deviance (similar to an ANOVA, but allowing the inverse variances of the estimated growth rates to be used as weights) using a GLM with a gaussian error model. Thus, growth rates of low variance received higher weight in statistical analysis than those with high variance. Growth rates across all species and by each species were compared between light treatments and presented as a box and whisker plot and interaction profile in Figures 1A and B respectively. The flagellate and colonial life stages of P. antarctica were considered to be functionally separate taxa due to the widely accepted physiological differences between these stages (eg. Lancelot et al. 1987, Marchant et al. 1991, Davidson & Marchant 1992b).

The size of 100 live cells of each species was measured and the mean cell volume calculated. Variation in the dimensions of cells fixed with Lugol's iodine from each light treatment were within 1 standard deviation of the live cell dimensions. The cell concentrations in replicates of each light treatment at each incubation time were pooled and the mean and standard error computed. Using the equations of Eppley et al. (1970) and the cell volume, carbon contributed by each species was then calculated. The carbon contributed by colonial stage P. antarctica was likely to be an underestimate as colony matrix was not considered in the calculation.

To investigate the minimum duration of UV exposure required to cause changes in phytoplankton species composition a 10 ml subsample was removed from each replicate of all light treatments after 2, 4, 6 and 8 days incubation and inoculated into 40 ml of sterile f/2 medium in 50 ml polystyrene culture flasks. Flasks were returned to culture maintenance conditions and grown for a further 9 days then thoroughly mixed and a 10 ml subsample removed and counted (as above). This procedure of subculturing samples after the various durations of exposure allowed expression and amplification of changes in phytoplankton species composition, while avoiding the effects of nutrient limitation in culture. For each incubation time and light treatment, the mean proportion of the

phytoplankton population contributed by each species following the 9 days amplification was calculated over 3 replicates . For these proportional data, error bars indicate ±1 standard error, calculated by arcsine square root transformation (after Zar 1984).

3. RESULTS AND DISCUSSION

Weather conditions were intermittently sunny during the eight days of exposure to natural Antarctic solar radiation. However, integrated UVA and UVB irradiances varied little during between-sample intervals (Table 1). Mean integrated UVA and UVB irradiance for each two day incubation period was $9.15 \pm 0.93 \times 10^5$ J.m^{-2} and $6.30 \pm 0.90\ 10^3$ J.m^{-2} respectively.

The analysis of deviance shows significant differences with species, light treatment and their interaction (Table 2). That species-specific growth rates are significantly different is not surprising. The distributions of growth rates across species for the different UV treatments are shown in Fig. 1A. Although the main effect due to UV treatment is significant (Table 2), the effects are not substantial, and the statistical significance arises largely because of a few cases where growth rate estimates with low variances have received a high weight in the analysis. The unweighted means and medians of the growth rates across species are not significantly different between light treatments. Similarly, no significant difference was found in total calculated cell carbon concentration between light treatments (Fig 2A). Thus, the overall growth and production by the community was maintained irrespective of light treatment.

In contrast, the interaction term is highly significant (Table 2). The changes in growth rates for at least some species under the different UV treatments demonstrates that over time, the species composition, in terms of cell concentration, will differ under the different UV regimes. Differences between the PAR and PAR + UVA light treatments, though significant ($P\{\chi^2<0.01\}$), were only slight. Exposure to UVB caused substantial changes in the growth rates (Fig 1B) which were highly statistically significant

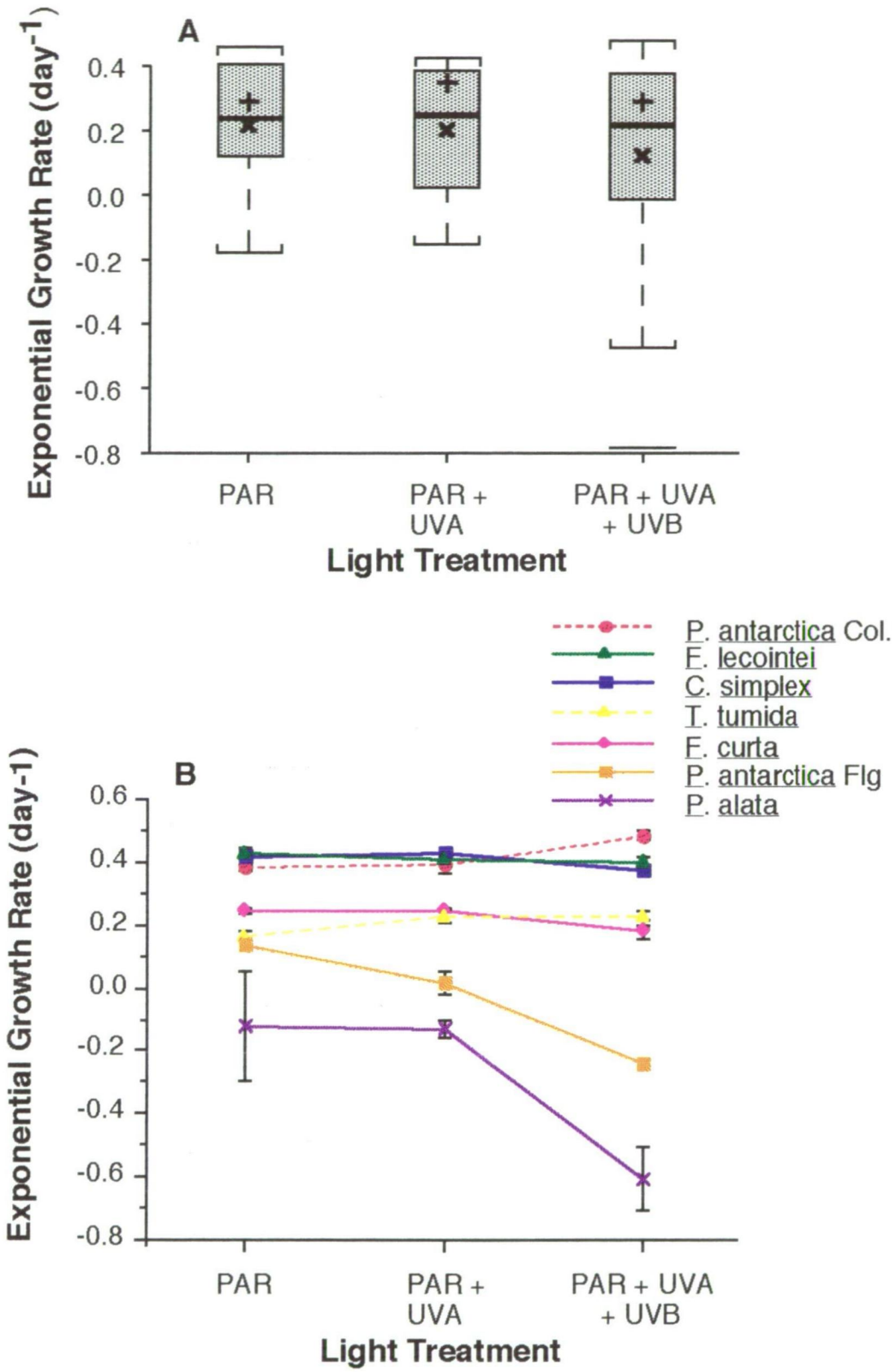


Fig. 1. Analysis of exponential growth rate (day-1) during 8 days exposure to natural irradiation. (A) Box and whisker plot showing similar growth rate across all species and comparing between light treatments and (B) interaction profile showing UVB-dependent changes in the growth rate of each species between light treatments with the standard error over three replicates. Box and whisker plot shows mean weighted (+), unweighted (x) and median (-) growth rates for all species between light treatments. Boxes enclose the interquartile range, whiskers extending to the standardised range and __ represents an outlier.

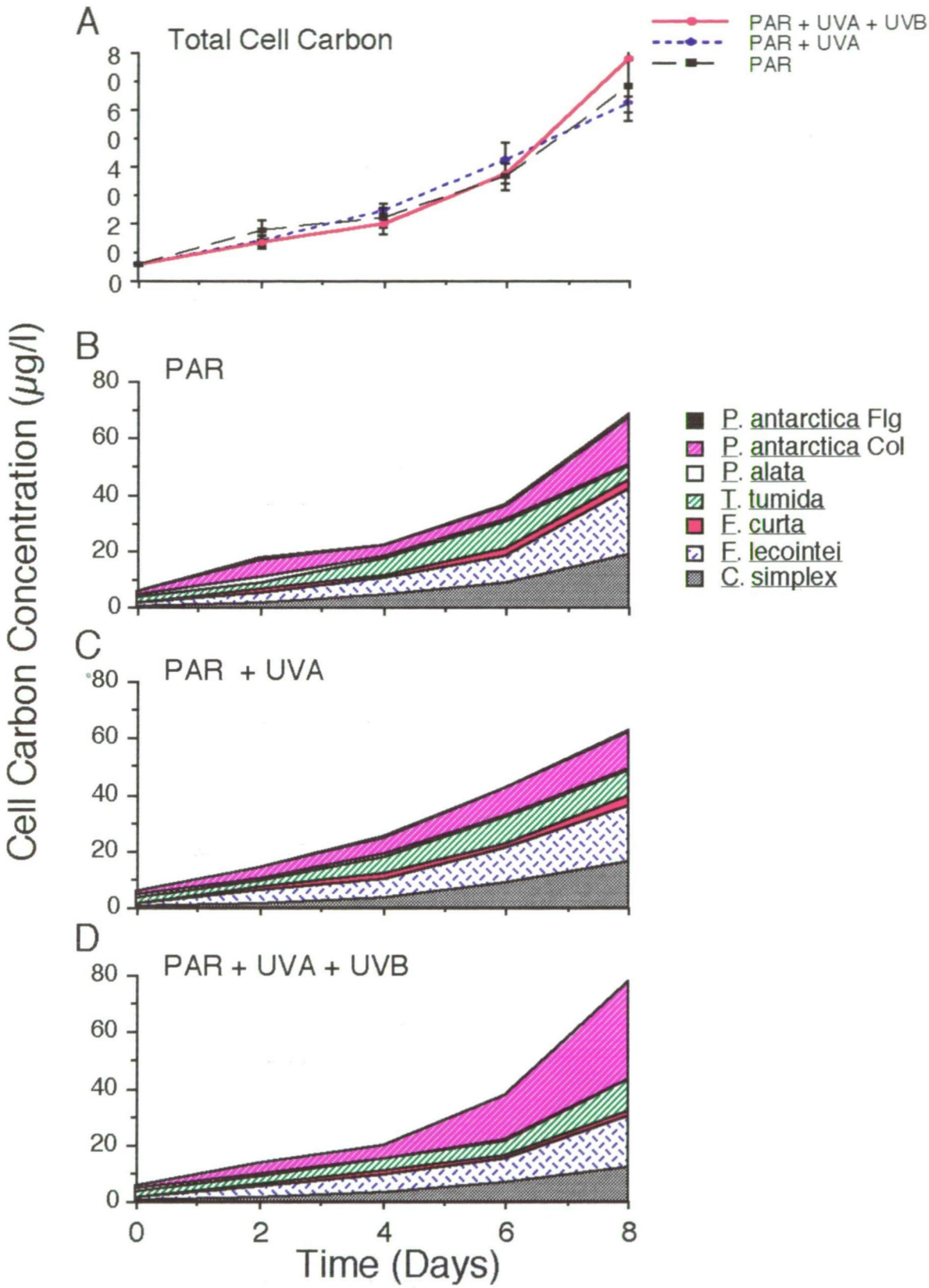


Fig. 2. Calculated cell carbon concentration of Antarctic phytoplankton during 8 days of natural irradiation showing (A) total cell carbon across all species and contributions by each species exposed to (B) PAR, (C) PAR + UVA and (D) PAR + UVA + UVB. P. antarctica Col and Flg indicate the colonial and flagellate life stage respectively. Error bars indicate standard error of the mean over three replicates.

($P\{\chi^2<0.000005\}$). The growth rate of diatoms and the flagellate stage of P. antarctica exposed to UVB either did not change significantly or declined. Only the colonial stage of P. antarctica showed a substantial promotion of growth as a result of exposure to UVB. The proportion of cell carbon contributed by diatoms and the flagellate stage of P. antarctica exposed to UVB also fell (Fig 2B-D) but were replaced by colonial stage P. antarctica. Thus, Antarctic near-surface UVB irradiance alters phytoplankton species composition in culture.

Table 1. Cumulative integrated UVA and UVB irradiance during 8 days natural irradiation measured using an International Light research radiometer and light sensors.

Days Irradiation	Cumulative Integrated UVA ($J.m^{-2}$)	Cumulative Integrated Erythemal UVB ($J.m^{-2}$)
0	0	0
2	9.75×10^5	6.48×10^3
4	18.43×10^5	12.26×10^3
6	28.51×10^5	19.74×10^3
8	36.59×10^5	25.18×10^3

The enhanced growth of colonial P. antarctica when exposed to UVB agrees with earlier findings which demonstrated that exposure of unialgal cultures of this species to in situ UVB increased cell size, growth rate and production by the colonial stage (Davidson & Marchant 1994) and may contribute to this species being one of the first to bloom in the ice and surface waters, where it frequently dominates the phytoplankton (Garrison et al. 1987, Fryxell & Kendrick 1988, Davidson & Marchant 1992a, b). Only P. alata exhibited no significant growth in the absence of UV radiation (Fig. 1B). This may reflect the observed sensitivity of the species to mechanical disturbance during subculturing which leads to an extended lag phase in its growth.

Table 2. Two way analysis of deviance table showing the significance of UV effects on the growth of Antarctic phytoplankton species. Df indicates degrees of freedom

Factor	Df	Deviance	Residual Df	Residual Deviance	Probability χ^2
Null			62	2809.445	
Species	6	2620.609	56	188.836	0.000000000
UV Treatment	2	11.630	54	177.206	0.00298
Species : UV Interaction	12	134.048	42	43.158	0.000000000

Our results differ from those reported by other authors. McMinn et al. (1994) found that sediment cores from fjords in East Antarctica did not exhibit evidence of a significant change in species composition of the diatom community since springtime ozone depletion began. However, it is likely that persistent sea ice, which attenuates UVB by at least 90% (Trodahl & Buckley 1989), provided shielding for these organisms. Bothwell et al. (1995) also criticise other aspects of the conclusions drawn by McMinn et al. (1994). Others (Smith et al. 1992, Karentz 1994, Karentz & Spero 1995) have reported that exposure of diatoms and P. antarctica to natural UVB did not alter their growth rates or that growth by P. antarctica declined.

This study used natural solar irradiance and selected Antarctic phytoplankton species but does not simulate natural Antarctic conditions. It also used a limited species assemblage grown in nutrient enriched media with and without exposure to a near-surface UVB light climate and wavelength structure. The effects of in situ UVB radiation on naturally occurring phytoplankton communities could differ from those I observed and the effect of increased UVB flux as a result of ozone depletion on phytoplankton species composition remain to be ascertained. Results obtained of changes in P. antarctica cell concentration with exposure to Antarctic solar irradiance by Karentz (1994) were highly variable due to the clumped distribution of cells in colonies. Changes within or between

light treatments were seldom significant and, unlike Smith et al. (1992) and Davidson and Marchant (1994), P. antarctica growth was also negative irrespective of light treatment. Differences between the results presented here and those of Smith et al. (1992) may be due to differences in methodology, the physiological state of cells or the use here of cultured material and a multi-species mix. Karentz and Spero (1995) report a strong positive correlation between P. antarctica concentration and column ozone concentration in the MIZ of the Bellingshausen Sea. The apparent conflict between their results and those presented here can only be reconciled with further study. Differences in methodology mean the studies are not directly comparable and it remains unclear whether the changes in P. antarctica concentration observed by Karentz and Spero (1995) were directly related to changes in the in situ UVB climate during their study.

The results presented in this chapter also contrasted with my previous findings. The colonial stage in the life cycle of P. antarctica produces high concentrations of UV–absorbing compounds (Marchant et al. 1991). These compounds enhanced survival of its colonial stage when exposed to high UVB irradiances (Marchant et al. 1991) but diatoms, which largely lack UV absorbing compounds, survived UVB irradiances 3 to 5 times that which caused mortality in colonial P. antarctica (Davidson et al. 1994). Thus, the role of UV–absorbing compounds in alleviating UVB damage is questionable. Many Antarctic marine organisms possess UV–absorbing compounds (Karentz et al. 1991b). However, the presumed protection afforded organisms by such compounds remains largely unquantified. Results presented here show that growth by the colonial stage of P. antarctica was promoted under natural UVB exposure. Consequently, the UVB irradiance at which P. antarctica died (Marchant et al. 1991) was not indicative of its enhanced growth at sub-lethal natural irradiances. Survival of diatoms to far higher UVB irradiances than P. antarctica (Davidson et al. 1994) was also not indicative of their slowed growth and production and the increased dominance of P. antarctica at natural, sublethal irradiances. The poor predictive value of a species response to high UVB irradiance experiments clearly demonstrates the limited value of extrapolating results of

such experiments (Worrest et al. 1978, Karentz et al. 1991a, Marchant et al. 1991, Davidson et al. 1994) to the natural environment.

Vernet et al. (1994) found that high haptophyte concentrations in Antarctic waters correlated with high in situ absorption at 330 nm and low inhibition of photosynthesis when exposed to UVB. At sub-lethal natural UVB irradiances, metabolic processes such as photosynthesis apparently are shielded from damage by UV–absorbing compounds. Other metabolic costs of exposure to UVB are thereby minimised. Thus, at natural UVB irradiances colonial P. antarctica may be afforded substantial protection by UV–absorbing compounds. High in situ absorption (Vernet et al. 1994) also suggests that blooms of P. antarctica may confer some UV protection on other organisms in the water column (Marchant et al. 1991); a feature not included in this experiment as colonial P. antarctica did not reach sufficient concentrations to attenuate UVB throughout the irradiated cultures.

The duration of UVB exposure required to elicit changes in phytoplankton species composition is critical in determining the potential magnitude of changes in phytoplankton species composition in Antarctic waters. Incubations of only 2 to 6 days may not have allowed expression of these changes in species composition. To express and amplify such changes, samples were removed from natural irradiation, subcultured, returned to culture maintenance conditions (which lack UV), and were grown for a further 9 days. Differences in proportional abundance of each species were not greatly increased by exposure times exceeding two days (Fig. 3). Thus, two days' exposure to ambient near-surface UVB irradiance was sufficient to largely determine the UVB-mediated species composition. Exposure to UVA and UVA + UVB increased the proportion of colonial P. antarctica in culture. The proportion contributed by diatoms, particularly C. simplex, declined (Fig 3A, B). The proportion of total cells contributed by other diatom species differed little between light treatments (Fig 3C-E). Two days was the shortest natural exposure time

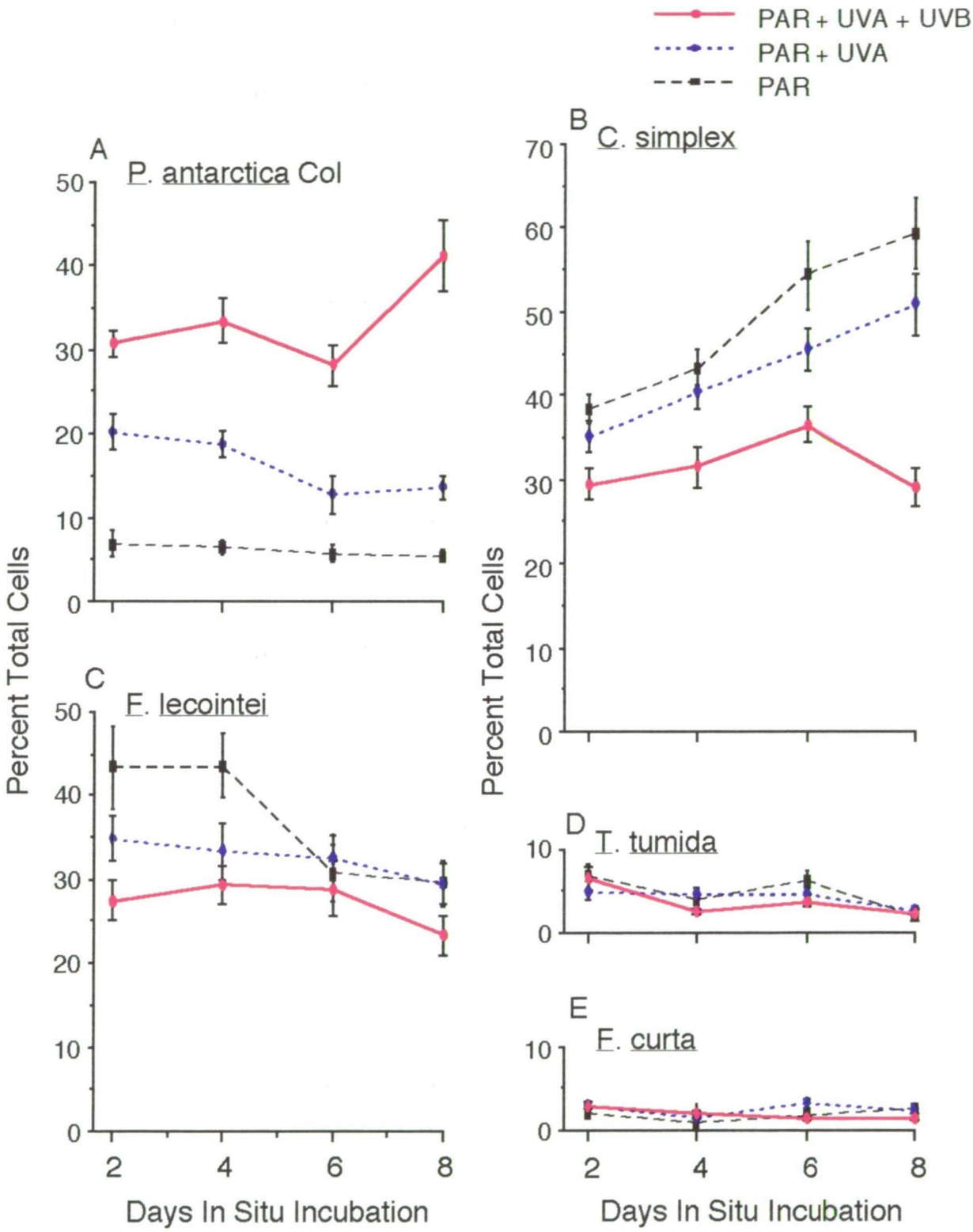


Fig. 3. Changes in the percent of total cell concentration contributed by each species following natural exposure for 2, 4, 6 and 8 days, subculturing and a further 9 days growth. (A) colonial stage P. antarctica (P. antarctica Col), (B) C. simplex, (C) F. lecointei, (D) T. tumida, (E) F. curta. P. alata and the flagellate stage P. antarctica together contributed < 2% of the total cell concentration and their proportion did not alter significantly over the incubation times. Results represent mean proportions of 3 replicates and error bars indicate standard error calculated by arcsine square root transformation after Zar (1984).

investigated; the minimum exposure required to elicit changes in species composition remains unknown.

Experimental evidence presented in this thesis strongly indicate that ozone depletion and the associated increase in UVB may promote the abundance of P. antarctica relative to diatoms in Antarctic waters. In conclusion, Chapter 9 investigates the effect of any increase in the absolute or relative abundance of this alga on the biotic and abiotic Antarctic marine environment.

CHAPTER 9

Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean.

1. INTRODUCTION

Depletion of stratospheric ozone over Antarctica now persists for most of the summer; that time of the year when sufficient light is available to support the proliferation of phytoplankton (Chapter 1, section 3). Resulting increases in the UVB irradiance have been demonstrated (Chapter 1, section 3). A wealth of scientific evidence shows that these wavelengths are damaging to plants and animals (Chapter 1, section 4 & 5). Biologically significant doses of UVB (280 - 320 nm) penetrate both the sea ice and the waters of the marginal ice zone (MIZ) (Chapter 1, section 6.1) surrounding Antarctica. The coincidence of stratospheric ozone depletion and near-surface seasonal algal blooms in the ice and marginal ice zone (MIZ) (Chapter 1, section 6.1) means that phytoplankton communities are likely to be exposed to increased UVB.

Phytoplankton and sea-ice algae are the foundation of the Antarctic food web. Any UVB–induced changes in their absolute or relative abundance may have important consequences for higher trophic levels (El-Sayed et al. 1990, Voytek 1990, Karentz 1991, Marchant et al. 1991, Davidson et al. in press). However, there is little direct evidence that increased UVB irradiance as a result of stratospheric ozone depletion has changed the absolute or relative abundance of phytoplankton in Antarctic waters (McMinn et al. 1994). The collapse of the Antarctic ecosystem predicted by El-Sayed et al. (1990) has not eventuated during the two decades since ozone depletion began. El-Sayed's prediction was based on simple experiments and, in hindsight, his conclusions regarding the effect of ozone depletion on Antarctic phytoplankton communities were an overreaction. However, they

focused substantial international scientific attention on the problem. Other authors have reported considerable reduction in survival, growth, production and changes in species composition (eg. Calkins & Thordardottir 1980, Karentz et al. 1991a, Smith et al. 1992, Behrenfeld et al. 1992, 1995, Karentz & Spero 1995, Davidson et al. in press). In contrast, Holm-Hansen et al. (1989b) suggested that significant impacts would only be felt in the upper water column and that vertical mixing would ameliorate the impacts of increased UVB on phytoplankton. Colleagues of Holm-Hansen (eg. Helbling et al 1994, Villafañe et al. 1995) and others (eg. Cullen & Lesser 1991) continue to present data indicating little effect of increased UVB on photosynthetic rates, long-term growth and production by Antarctic phytoplankton. Despite over a decade of burgeoning scientific attention and a great many publications, the impact of increased UVB radiation on Antarctic marine phytoplankton remains unsure.

2. THE COMPARATIVE UVB PHOTOBIOLOGY OF Phaeocystis antarctica AND SELECTED ANTARCTIC MARINE DIATOMS

The measured impact of UVB on phytoplankton varies greatly between studies (Chapter 1, section 6.1) and considerable research is required to determine the effect of stratospheric ozone depletion on phytoplankton. Many authors propose that the likely result of increased UVB in Antarctic waters is a shift in species composition favouring those species with greater tolerance of UVB (Davidson et al. 1996). The high lethal limits of UVB exposure of some species (Davidson et al. 1994) but subtle species specific differences in photobiology at sublethal UVB irradiances (Davidson & Marchant 1994, Davidson et al. 1996) presented in this thesis support such a proposal.

Phaeocystis antarctica possesses UV–absorbing compounds that enhance its survival when exposed to UVB (Marchant et al. 1991). Diatoms, which largely lack these compounds, survive much higher irradiances than P. antarctica (Davidson et al. 1994) and were able to survive and sustain growth during and after exposure to in situ Antarctic UVB. However, when exposed to natural near–surface Antarctic UVB irradiances P.

antarctica increased its growth, cell size and production (Davidson & Marchant 1994), and outcompeted the diatoms species examined (Davidson et al. 1996). Thus, the UVB tolerance determining survival of an organism need not indicate its response to sub-lethal irradiances.

Results indicate that UVB radiation can be beneficial to some phytoplankton. Low UVB irradiances apparently enhanced survival of the diatom species examined and may be involved in repair of UVA–induced damage (Davidson et al. 1994). Furthermore, Phaeocystis antarctica benefited from exposure to UVB (Davidson et al. 1994, Davidson et al. 1996). The reason for this remains obscure but may be due to absorption by chlorophyll a of fluorescence emissions from the UV–absorbing compounds, thereby benefiting from the high energy of UVB photons (Kawaguti 1969, Silvalingham et al. 1976)

Caveats must be placed on the experimental findings in this thesis. The experiments did not include such factors as vertical mixing, trophic interactions or nutrient concentrations. Nor did they include the multiplicity of phytoplankton species inhabiting the Southern Ocean. They did, however, use or simulated natural Antarctic irradiances, measured responses over ecologically sustainable time scales, and included key contributors to the Antarctic phytoplankton community. While cognizant of the experimental limitations, these results indicate that increased UVB is likely to increase the abundance of P. antarctica relative to Antarctic marine diatoms.

3. GRAZING ON Phaeocystis antarctica

The most abundant components of the phytoplankton in the ice and MIZ are diatoms, principally of the genera Nitzschia and Fragilariopsis, and P. antarctica (Chapter 2 & 3). Phaeocystis blooms are utilized by protozoa (Fryxell et al. 1984, Admiraal & Venekamp 1986, Lutter et al. 1989, Wassmann et al. 1990, Weisse & Scheffel-Möser 1990, Davidson & Marchant 1992). However, although Phaeocystis antarctica is grazed by

herbivores including Euphausia superba (Sieburth 1960, Marchant & Nash 1986, Virtue et al. 1993b), the impact of grazing on populations of this alga and its food value are equivocal (Chapter 3, section 5.3). Phaeocystis is poorly, inefficiently, or incapable of being grazed by metazoa. It is also avoided by invertebrates and fish, of low nutritional value, and does not support copepod growth and reproduction (Chapter 3, section 5).

In an investigation of the impact of copepod grazing Phaeocystis comprised about 97% of the phytoplankton biomass. However, diatoms, which comprised most of the remaining 3%, accounted for some 74% of the copepod diet (Claustre et al. 1990). Only 1.5% of the biomass of Phaeocystis was grazed by the copepods, the remainder apparently being lost to the pelagic food web. Phaeocystis, including P. antarctica (Priscu et al. 1990, Virtue et al. 1993b), is of poor nutritional value; containing lower concentrations of polyunsaturated fatty acids, neutral lipids, essential fatty acids and vitamin C than has been found in diatoms (Chapter 3, section 5.3). Phaeocystis may also change in particle size over three orders of magnitude between the flagellate stage and colonial life stages and releases dimethyl sulfide (DMS) (Chapter 3, section 6.8), acrylic acid (Chapter 3, section 6.9) and large quantities of mucilaginous carbohydrate (Chapter 3, section 6.5). These physical and chemical characteristics apparently deter grazers and Antarctic euphausiids reportedly exhibit a dietary preference for diatoms (Meyer & El-Sayed 1983, Miller & Hampton 1989). Utilization of Phaeocystis blooms by microheterotrophs and the "microbial loop" may form an important link with higher trophic levels (Chapter 3, section 5.4, Chapter 4). However, very little of the carbon attributable to Phaeocystis antarctica is apparently utilized by metazoa (Chapter 4) and, as was found by Claustre et al. (1990), most of the carbon was not used in situ.

4. VERTICAL CARBON FLUX IN THE MARGINAL ICE ZONE

Massive deposits of diatomaceous ooze in Southern Ocean sediments are dominated by those diatom taxa found in the MIZ (Truesdale & Kellogg 1979). Sedimentation is apparently the principal fate of much of the ice edge bloom (Smith & Nelson 1986,

Bodungen et al. 1986, Fischer et al. 1988). A substantial amount of the phytoplankton production sinks rapidly from the euphotic zone and contributes directly to deep carbon flux (Smith & Nelson 1986). In addition, faecal pellets of microheterotrophs (Nöthig & von Bodungen 1989, Buck et al. 1990) and metazoa (Wefer et al. 1988), also contribute substantially to particulate carbon flux from surface waters of the Southern Ocean. In contrast to the marked seasonality of the sedimentation of primary producers and the faeces of grazers, cast exoskeletons of Euphausia superba are likely to constitute a major year-round flux of particulate organic carbon from the euphotic zone to deep water or the sediments (Nicol & Stolp 1989).

Phaeocystis apparently contributes little to the direct flux of carbon to the deep ocean (Chapter 3, section 5.5). Some authors report that Phaeocystis colonies are positively buoyant (Skreslet 1988, Riebesell 1993). In addition, this alga also demonstrates little potential to aggregate; a process that can increase sinking rate (Riebesell 1993). Thus, Phaeocystis blooms characteristically sink slowly (Wassmann 1994). Instead of sedimenting, much of the carbon is respired by microheterotrophs and bacteria in the upper 100 m of the water column (Chapter 3, section 5.5). Avoidance of this alga by metazoan grazers would also mean it contributes little to vertical carbon flux in the form of faeces and moults. Further, organisms of the microbial loop are more likely to produce smaller, slower sinking particles than metazoan faeces and moults (Marchant & Davidson 1991). Thus, utilization of Phaeocystis blooms by microheterotrophs would constitute a lesser carbon flux to deep water than the larger faster sinking material from metazoa.

5. Phaeocystis antarctica AND DIMETHYL SULFIDE PRODUCTION.

Phaeocystis is reportedly the principal producer of dimethyl sulfide (DMS) (Chapter 3, section 6.8). Production of DMS and its precursor dimethylsulfoniopropionate (DMSP) by Phaeocystis may account for as much as 20% of its photoassimilated carbon (Matrai et al. 1995) and elevated concentrations of DMS in the water column, particularly in Antarctic waters, correlate with high concentrations of this alga (Chapter 3, section 6.8).

In Antarctic waters, Gibson et al. (1990) estimate that Phaeocystis antarctica may contribute as much as 10% of the total global flux of DMS to the atmosphere. Oxidation of this DMS forms sulfate particles which constitute a major source of cloud condensation nuclei (CCN). Bates et al. (1987a) and Charlson et al. (1987) propose that the abundance of CCN determines global albedo thereby establishing a mechanism for the regulation of climate by marine biological activity.

6. CONSEQUENCES OF INCREASED RELATIVE ABUNDANCE OF Phaeocystis antarctica

The results presented in this thesis indicate that stratospheric ozone depletion over Antarctica and the consequent rise in UVB irradiance will change phytoplankton species composition, increasing the relative abundance of P. antarctica. Few data are available to indicate the consequences of such a change in species dominance and the effects proposed in Fig. 1 are speculative. However if, as appears to be the case, crustacea selectively graze diatoms in preference to Phaeocystis and diatoms are of greater food value, then there is the possibility that populations of krill and other grazers could be nutrient limited with a consequent decline in food availability to higher trophic levels. Reduced availability of more relatively nutritious food may also reduce the fecundity of grazers (Verity & Smayda 1989).

Any diminution in diatom growth is likely to reduce vertical carbon flux. In addition to the reduced flux of faeces and moults of grazers that prefer diatoms there would be a decline in the flux of diatoms themselves. The high concentrations of slow sinking POC and DOC produced by Phaeocystis antarctica provide substrates for bacteria and microheterotrophs in surface waters (Davidson & Marchant 1992). Respiration by these organisms is likely to result in higher concentrations of CO_2 in the photic zone. Thus, increased dominance of P. antarctica could reduce the transfer of atmospheric carbon to the deep ocean, exacerbating the accumulation of greenhouse CO_2 in the atmosphere.

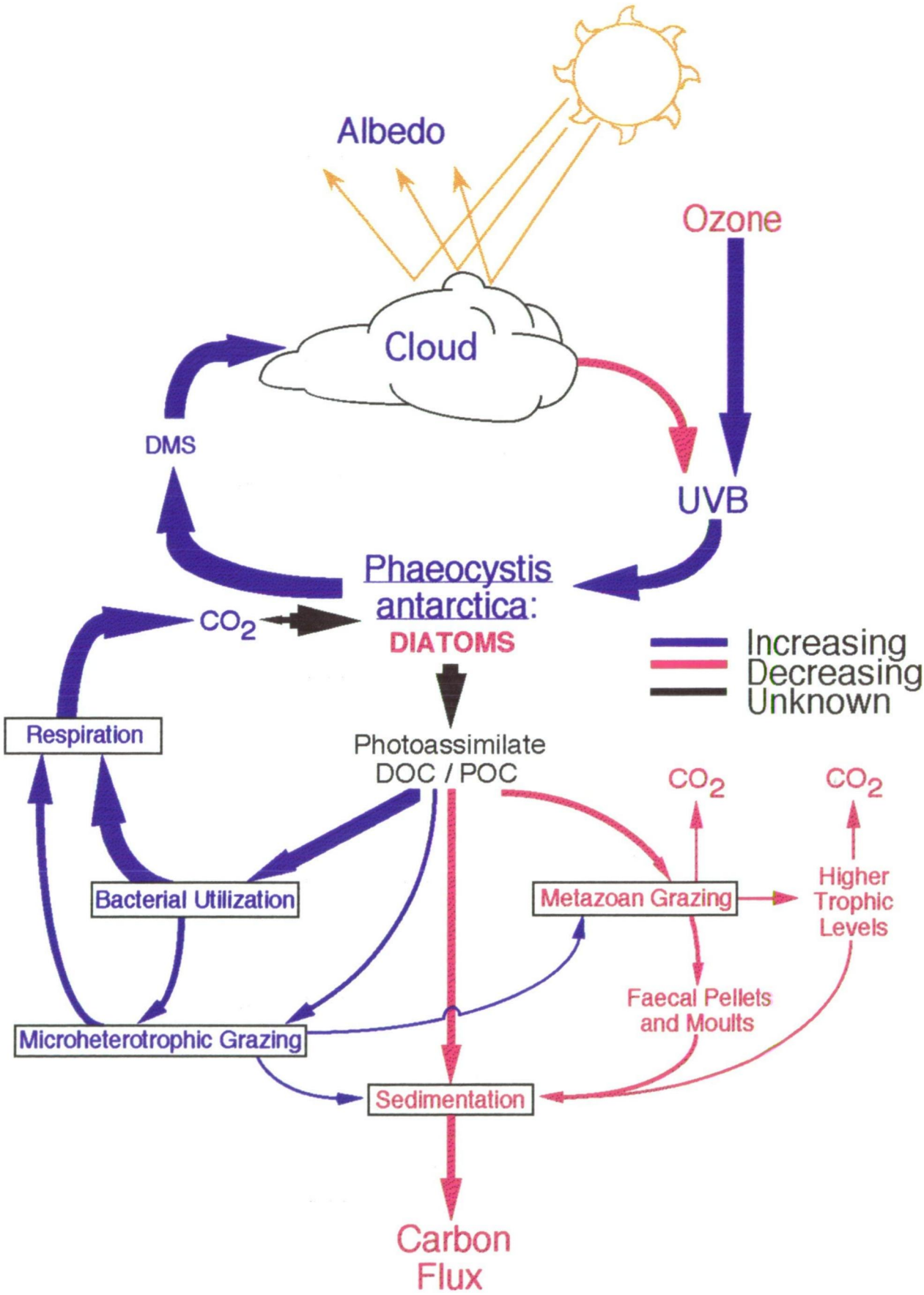


Fig. 1. Schematic drawing giving some of the possible effects of a UVB-induced increase in the relative abundance of Phaeocystis antarctica on grazing, carbon flux and DMS production in Antarctic waters. Processes are framed by rectangles, stocks are not.

The results of Davidson et al. (1996) show an 11% increase in Phaeocystis antarctica concentration as a result of 2 days exposure to natural Antarctic UVB. UV-B in spring and during the summer solstice has increased by 50-100% as a result of ozone depletion (Frederick & Lubin 1994). Thus, assuming half the 11% increase in P. antarctica is due to increased UVB radiation resulting from ozone depletion, 5.8×10^{12} g, or around 1.7% of carbon previously fixed annually by diatoms in the MIZ, would be replaced by P. antarctica (after Smith et al. 1992). If 20% of the carbon sequestered by diatoms is lost to deep water (IPCC 1944) and none is contributed by the increased proportion of P. antarctica in the MIZ alone, the ozone-induced increase in the relative abundance of P. antarctica would cause a decline in deep ocean carbon flux of 1.16×10^{12} g or 0.06% (IPCC 1944) of the annual net uptake of the world's oceans.

Any UVB-mediated increase in the relative abundance of P. antarctica could alter the Antarctic food web by changing the particle size, form, availability and nutritional value of carbon to higher trophic levels (Marchant & Davidson 1991). It could also effect climate through changes in global albedo (Charlson et al. 1987). Thus, in Antarctic waters we see the nexus of two burning issues in man's degradation of the global environment; the interconnection of ozone depletion and global warming.

REFERENCES

Admiraal W, Veldhuis MJW (1987) Determination of nucleosides and nucleotides in seawater by HPLC; application to the phosphatase activity in cultures of the alga Phaeocystis pouchetii. Mar Ecol Prog Ser 36: 277-285

Admiraal W, Venekamp LAH (1986) Significance of tintinnid grazing during blooms of Phaeocystis pouchetii (Haptophyceae) in Dutch coastal waters. Neth J Sea Res 20: 61-66

Ainley DG, Fraser WR, Sullivan CW, Torres JJ, Hopkins TL, Smith WO Jr(1986) Antarctic mesopelagic micronekton: evidence from seabirds that pack ice affects community structure. Science 232: 847-849

Al-Hasan RH, Ali AM, Radwan SS (1990) Lipids, and their constituent fatty acids, of Phaeocystis sp. from the Arabian Gulf. Mar Biol 105: 9-14

Andersson B, Salter AH, Virgin I, Vass I, Styring S (1992) Photodamage to photosystem II - primary and secondary events. J Photochem Photobiol B Biol 15: 15-31

Andreae MO (1980) Dimethyl sulfoxide in marine and freshwaters. Limnol Oceanogr 25: 1054-1063

Andreae MO, Raemdonck H (1983) Dimethyl sulfide in the surface ocean and the marine atmosphere: a global view. Science 221: 744-747

Armonies W (1989) Occurrence of meiofauna in Phaeocystis seafoam. Mar Ecol Prog Ser 53: 305-309

Ashworth TK, Prasad AKSK, Fryxell GA (1990) AMERIEZ 88: Phytoplankton distribution across the Weddell Sea ice edge during austral winter. Eos 71: 105

Atkinson RJ, Matthews WA, Newman PA, Plumb RA (1989) Evidence of the mid-latitude impact of Antarctic ozone depletion. Nature 340: 290-294

Atkinson LP, Paffenhöfer G-A, Dunstan WM (1978) The chemical and biological effect of a Gulf Stream intrusion off St. Augustine, Florida. Bull Mar Sci 28: 667-679

Badour SS (1968) Experimental separation of cell division and silica shell formation in Cyclotella cryptica. Microbiol 62: 17-33

Baker KS, Smith RC, Green AES (1980) Middle ultraviolet radiation reaching the ocean surface. Photochem Photobiol 32: 367-374

Balech E, El-Sayed SZ (1965) Microplankton of the Weddell Sea. In: Llando GA (ed) Biology of the Antarctic seas II. vol 5, National Academy of Science, National Research Council, Washington, DC, p 109-127

Barnard WR, Andreae MO, Iverson RL (1984) Dimethylsulfide and Phaeocystis pouchetii in the southeastern Bering Sea. Cont Shelf Res 3: 103-113

Bartsch A, Dieckmann (1988) Variability and growth of algae overwintering in Antarctic sea ice. In: SCAR fifth symposium on Antarctic biology, Hobart, Australia, 29th August - 3rd September 1988, p 7

Bates TS, Charlson RJ, Gammon RH (1987a) Evidence for the climatic role of marine sulphur. Nature 329: 319-321

Bates TS, Cline JD, Gammon RH, Kelly-Hansen SR (1987b) Regional and seasonal variation in the flux of oceanic dimethylsulfide to the atmosphere. J Geophys Res 93: 2930-2938

Bätje M, Michaelis H (1986) Phaeocystis pouchetii blooms in the East Frisian coastal waters (German Bight, North Sea). Mar Biol 93: 21-27

Baumann MEM, Brandini FP, Staubes (1993) The influence of light and temperature on carbon-specific DMS release by cultures of Phaeocystis antarctica and three antarctic diatoms. Mar Chem 44, 129-136

Baumann MEM, Jahnke J (1986) Marine planktonalgen der Arktis; Die Haptophycee Phaeocystis pouchetii. Mikrokosmos 75: 262-265

Baumann MEM, Lancelot C, Brandini FP, Sakshaug E, John DM (1994) The taxonomic identity of the cosmopolitan prymnesiophyte Phaeocystis: a morphological and ecophysiological approach. J Mar Syst 5: 5-22

Bautista B, Harris R P, Rodriguez V, Guerrero F (1994) Temporal variability in copepod fecundity during two different spring bloom periods in coastal waters off Plymouth (SW England). J Plankton Res 16: 1367-1377

Bautista B, Harris RP, Tranter PRG, Harbour D (1992) In situ copepod feeding and grazing rates during a spring bloom dominated by Phaeocystis sp. in the English Channel. J Plankton Res 14: 691-703

Behrenfeld MJ, Chapman JW, Hardy JT, Lee II H (1993) Is there a common response to ultraviolet-B radiation by phytoplankton. Mar Ecol Prog Ser 102: 59-68

Behrenfeld MJ, Hardy JT, Lee II H (1992) Chronic effects of ultraviolet-B radiation on growth and cell volume of Phaeodactylum tricornutum (Bacillariophyceae). J Phycol 28: 757-760

Behrenfeld MJ, Lean DRS, Lee II H (1995) Ultraviolet-B radiation effects on inorganic nitrogen uptake by natural assemblages of oceanic plankton. J Phycol 31: 25-36

Bidigare RR (1989) Potential effects of UV-B radiation on marine organisms of the Southern Ocean: distributions of phytoplankton and krill during austral spring. Photochem Photobiol 50: 469-477

Bidigare RR, Frank TJ, Zastrow C, Brooks JM (1986) The distribution of algal chlorophylls and their degradation products in the Southern Ocean. Deep-Sea Res 33: 923-937

Billen G, Becquevort S (1991) Phytoplankton-bacteria relationships in the Antarctic marine ecosystem. Polar Res 10: 245-255

Billen G, Fontigny A (1987) Dynamics of a Phaeocystis-dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar Ecol Prog Ser 37: 249-257

Billen G, Lancelot C, Mathot S (1987) Ecophysiology of phyto- and bacterioplankton growth in the Prydz Bay area during the austral summer 1987. II. Bacterioplankton activity. In: Proceedings Belgian National Colloquium on Antarctic Research. Prime Ministers Services - Service Policy Office, Brussels, p 133-146

Bird DF, Karl DM (1991) Massive prasinophyte bloom in the northern Gerlache Strait. Antarct J US 26: 152-154

Bjørnland T, Guillard RRL, Liaaen-Jensen S (1988) Phaeocystis sp. clone 677–3 - a tropical marine planktonic prymnesiophyte with fucoxanthin and 19'-acyloxyfucoxanthins as chemosystematic carotenoid markers. Biochem Syst Ecol 16: 445-452

Bjørnsen PK, Kuparinen J (1991) Growth and herbivory by heterotrophic dinoflagellates in the Southern Ocean, studied by microcosm experiments. Mar Biol 109: 397-405

Boalch GT (1984) Algal blooms and their effect on fishing in the English Channel. Proceedings of the XI international Seaward symposium. Hydrobiologia 116/117 449-452

Boalch GT (1987) Recent blooms in the western English Channel. Rapp P -v Réun Cons Perm Int Explor Mer 187: 94-97

Boalch GT, Harbour DS (1977) Unusual diatom off the coast of south-west England and its effect on fishing. Nature 269: 687-688

Bölter M, Dawson R (1982) Heterotrophic utilisation of biochemical compounds in Antarctic waters. Neth J Sea Res 16: 315-332

Booth BC, Lewin J, Norris RE (1982) Nanoplankton species predominant in the subarctic Pacific in May and June 1978. Deep-Sea Res 2: 185-200

Booth CR, Madronich S (1994) Radiation amplification factors: improved formulation accounts for large increases in ultraviolet radiation associated with Antarctic ozone depletion. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 39-42

Booth BC, Marchant HJ (1987) Parmales, a new order of marine chrysophytes, with descriptions of three new genera and seven new species. J Phycol 23: 245-260

Bothwell ML, Karentz D, Carpenter EJ (1995) No UVB effect? Nature 374: 601

Bothwell ML, Sherbot D, Pollock CM (1994) Ecosystem response to solar ultraviolet-B radiation: influence of trophic level interactions. Science 265: 97-100

Bothwell ML, Sherbot D, Roberge AC, Daley RJ (1993) Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: short-term versus long-term effects. J Phycol 29: 24-35

Bougard M (1979) Étude bibliographique sur le phytoflagellate Phaeocystis. Institut de Biologie Maritime et Regionale de Wimereau, Université des Sciences et Technique de Lille, p 1-30

Bourrelly P (1957) Researches sur les Chrysophycees: morphologie, phylogenie, systematique. Rev Algol, Mém H-Sér 1: 1-42

Bradstock M, Mackenzie L (1981) The Tasman Bay slime story. Catch '81 December, 29-30

Brandini FP (1993) Phytoplankton biomass in an Antarctic coastal environment during stable water conditions – implications for the iron limitation theory. Mar Ecol Prog Ser 93: 267-275

Brandini FP, Kutner MBB (1987) Phytoplankton and nutrient distributions off the northern South Shetland Islands (summer 1984 - BIOMASS/SIBEX). La Mer 25: 93-103

Brasseur G (1987) The endangered ozone layer. Environment 29: 6-45

Brimblecombe P, Shooter D (1986) Photo-oxidation of dimethylsulphide in aqueous solution. Mar Chem 19: 343-353

Brussaard CPD, Riegman R, Noordeloos AAM, Cadée GC, Witte H, Kop AJ, Nieuwland G,van Duyl FC, Bak RPM (1995) Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar Ecol Prog Ser 123: 259-271

Buck KR, Bolt PA, Garrison DL (1990) Phagotrophy and fecal pellet production by an athecate dinoflagellate in Antarctic sea ice. Mar Ecol Prog Ser 60: 75-84

Buck KR, Garrison DL (1983) Protists from the ice-edge region of the Weddell Sea. Deep-Sea Res 30: 1261-1277

Buck KR, Garrison DL (1988) Distribution and abundance of choanoflagellates (Acanthoecidae) across the ice edge zone in the Weddell Sea, Antarctica. Mar Biol 98: 263-269

Bühlmann B, Bossard P, Uehlinger U (1987) The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J Plankton Res 9: 935-943

Buma AJG, Gieskes WWC, Thomsen HA (1992) Abundance of Cryptophyceae and chlorophyll b–containing organisms in the Weddell-Scotia confluence area in the spring of 1988. Polar Biol 12: 43-52

Bunt JS, Wood EJF (1986) Microalgae in Antarctic sea ice. Nature 199: 1254-1255

Burkholder PR, Mandelli EF (1965) Productivity of Antarctic microalgae in Antarctic sea ice. Science 149: 872-874

Burkholder PR, Sieburth JMcN (1961) Phytoplankton and chlorophyll in the Gerlache and Bransfield Straits of Antarctica. Limnol Oceanogr 6: 45-52

Burns NM, Rosa F (1980) In situ measurements of the settling velocity of organic carbon particles and ten species of phytoplankton. Limnol Oceanogr 2: 855-864

Burton GW, Ingold KU (1984) β-carotene: an unusual type of lipid antioxidant. Science 224: 569-573

Cadée GC (1982) Tidal and seasonal variation in particulate and dissolved organic carbon in the western Dutch Wadden Sea and Marsdiep tidal inlet. Neth J Sea Res 15: 228-249

Cadée GC (1986) Increased phytoplankton primary production in the Marsdiep area (western Dutch Wadden Sea). Neth J Sea Res 20: 285-290

Cadée GC (1991) Long-term changes in phytoplankton in marine coastal waters (abstract). J Phycol 27: 12

Cadée GC, Hegeman J (1974) Primary production of phytoplankton in the Dutch Wadden Sea. Neth J Sea Res 8: 240-259

Cadée GC, Hegeman J (1979) Phytoplankton primary production, chlorophyll and composition in an inlet of the western Wadden Sea (Marsdiep). Neth J Sea Res 13: 224-241

Cadée GC, Hegeman J (1986) Seasonal and annual variation in Phaeocystis pouchetii (Haptophyceae) in the westernmost inlet of the Wadden Sea during the 1973 to 1985 period. Neth J Sea Res 20: 29-36

Caldwell MM (1981) Plant responses to solar ultraviolet radiation. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Encyclopedia of plant physiology. New Series, Physiological Plant Ecology. 1, Springer, New York, p 169-197

Calkins J (1982) The role of solar ultraviolet radiation in marine ecosystems. Plenum Press, New York

Calkins J, Thordardottir T (1980) The ecological significance of solar UV radiation on aquatic organisms. Nature 283: 563-566

Cariou V, Casotti R, Birrien J-L, Vaulot D (1994) The initiation of Phaeocystis colonies. J Plankton Res 16: 457-470

Carreto J I, Carignan MO, Daleo G, De Marco SG (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate Alexandrium excavatum: UV-photoprotective compounds? J Plankton Res 12: 909-921

Chambers JM, Hastie TJ (1993) Statistical models in S. Chapman and Hall, London

Chang FH (1983) The mucilage–producing Phaeocystis pouchetii (Prymnesiophyceae) cultured from the 1981 "Tasman Bay Slime". NZ J Mar Freshw Res 17: 165-168

Chang FH (1984) The ultrastructure of Phaeocystis pouchetii (Haptophyceae) vegetative colonies with special reference to the production of new mucilagenous envelope. NZ J Mar Freshw Res 18: 303-308

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. Nature 326: 655-661

Chioccara F, Della Gala A, de Rosa M, Novellino E, Prota G (1979) Occurrence of two new mycosporine–like amino acids, mytilins A and B, in the edible mussel, Mytilis galloprovincialis. Tetrahedron Lett 34: 3181-3182

Chioccara F, Della Gala A, de Rosa M, Novellino E, Prota G (1980) Mycosporine amino acids and related compounds from the eggs of fishes. Bull Soc Chim Belg 89: 1101-1106

Chu S.P. (1946) The utilization of organic phosphorus by phytoplankton. J Mar Biol Ass UK 26: 285-295

Clarke DB, Ackerly SF (1984) Sea ice structure and biological activity in the Antarctic marginal ice zone. J Geophys Res 89: 2087-2095

Claustre H, Marty J-C, Cassiani L (1989) Intraspecific differences in the biochemical composition of a diatom during a spring bloom in Villefranche-sur-Mer Bay, Mediterranean Sea. J Exp Mar Biol Ecol 129: 17-32

Claustre H, Poulet SA, Williams R, Marty J-C, Coombs S, Ben Mlih F, Hapette AM, Martin-Jezequel V (1990) A biochemical investigation of a Phaeocystis sp. bloom in the Irish Sea. J Mar Biol Ass UK 70: 197- 207

Cloud PE Jr (1968) Atmospheric and hydrospheric evolution on primative Earth. Science 160: 729-736

Codispoti LA, Friederich GE, Whaling P, Friebertshauser ME (1990) Some implications of the nutrient observations made during the 1989 CEAREX experiment. Eos 71: 79

Colijn F (1983) Primary production in the Ems-Dollard Estuary. Ph. D. Thesis, University of Groningen, p 1-123

Colijn F, Villerius L, Radmaker M, Hammer KD, Eberlein K (1990) Changes in spatial distribution of primary production, photosynthetic pigments and phytoplankton species composition during two surveys in the German Bight. Neth J Sea Res 25: 155-164

Crocker KM, Ondrusek ME, Petty RL, Smith RC (1995) Dimethyl sulfide, algal pigments and light in an Antarctic Phaeocystis sp. bloom. Mar Biol 124: 335-340

Crutzen PJ (1992) Ultraviolet on the increase. Nature 356: 104-105

Cullen JJ, Lesser MP (1991) Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar Biol 111: 183-190

Cullen JJ, Neale PJ, Lesser MP (1992) Biological weighting functions for the inhibition of phytoplankton photosynthesis by ultraviolet radiation. Science 258: 646-650

Culotta E (1994) UV-B effects: bad for insect larvae means good for algae. Science 265: 30

Dagg MJ, Vidal J, Whitledge TE, Iverson RL, Goering JJ (1982) The feeding, respiration and excretion of zooplankton in the Bering Sea during a spring bloom. Deep-Sea Res 29: 45-63

Damkaer DM, Dey DB (1983) UV damage and photoreactivation potentials of the larval shrimp, Pandalus platyceros, and adult euphausiids, Thysanoessa raschii. Oecologia 60: 169-175

Daro MH (1985) Field study of selectivity, efficiency and daily variation in the feeding of the marine copepod Temora longicornis, in the Southern Bight of the North Sea. Bull Mar Sci 37: 764

Davidson AT (1985) Aspects of the biology of Phaeocystis pouchetii (Prymnesiophyceae). Hons. Thesis, University of Tasmania

Davidson AT, Bramich D, Marchant HJ, McMinn A (1994) Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms. Mar Biol 119: 507-515

Davidson AT, Marchant HJ (1987) Binding of manganese by antarctic Phaeocystis pouchetii and the role of bacteria in its release. Mar Biol 95: 481-487

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

Davidson AT, Marchant HJ (1992b) The biology and ecology of Phaeocystis (Prymnesiophyceae). In: Round FE, Chapman DJ (eds) Progress in phycological research. Biopress, Bristol, 8: 1-45.

Davidson AT, Marchant HJ (1992c) The impact of ultraviolet radiation on Phaeocystis and selected species of Antarctic marine diatoms. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 43-52

Davidson AT, Marchant HJ (1994) The in situ photobiology of Antarctic Phaeocystis and selected diatom species. Polar Biol 7: 53-69

Davidson AT, Marchant HJ, de la Mare WK (1996) Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culture. Aquat Microb Ecol 10: 299-305

Davies AG, de Madariaga I, Bautista B, Fernández E, Harbour DS, Serret P, Tranter PRG (1992) The ecoology of a coastal Phaeocystis bloom in the north-western English Channel in 1990. J Mar Biol Ass UK 72: 691-708

Demmig B, Winter K, Krüger A, Cyzgan F-C (1987) Photoinhibition and zeaxanthin in intact leaves - a possible role for the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol 84: 218-224

Denman KL, Gargett AE (1983) Time and space scales of vertical mixing and advection of phytoplankton in the upper ocean. Limnol Oceanogr 28: 801-815

Deprez PP, Franzmann PD, Burton HR (1986) Determination of reduced sulfur gases in Antarctic lakes and seawater by gas chromatography after solid absorbant preconcentration. J Chromatogr 362: 9-21

Dey DB, Damkaer DM, Heron GA (1988) UV-B dose/dose-rate responses of seasonally abundant copepods of Puget Sound. Oecologia 76: 321-329

Dodge JD, Priddle J (1987) Species composition and ecology of dinoflagellates from the Southern Ocean near South Georgia. J Plankton Res 9: 685-697

Döhler G (1984) Effect of UV-B radiation on the marine diatoms Lauderia annulata and Thalassiosira rotula grown in different salinities. Mar Biol 83: 247-253

Döhler G (1985) Effect of UV-B radiation (290 - 320 nm) on the nitrogen metabolism of several diatoms. J Plant Physiol 118: 391-400

Döhler G (1987) Effect of irradiation on nitrogen metabolism in marine diatoms and phytoplankton. Oceanis 13: 487-493

Döhler G (1992) Impact of UV-B radiation on uptake of ^{15}N–ammonia and ^{15}N–nitrate by phytoplankton of the Wadden Sea. Mar Biol 112: 485-489

Döhler G, Biermann T (1994) Impact of UV-B radiation on the lipid and fatty acid composition of synchronized Ditylum brightwellii (West) Grunow. Naturforsch 49: 607-614

Döhler G, Hagmeier E, Grigoleit E, Krause KD (1991) Impact of solar UV radiation on uptake of ^{15}N–ammonia and ^{15}N–nitrate by marine diatoms and natural phytoplankton. Biochem Physiol Pflanz 187: 293-303

Döhler G, Worrest RC, Biermann I, Zink J (1987) Photosynthetic $^{14}CO_2$ fixation and ^{15}N-ammonia assimilation during UV-B radiation of Lithodesmium variable. Physiologia Pl 70: 511-515

Dunlap WC, Chalker BE (1986) Identification and quantitation of near–UV absorbing compounds (S-320) in hermatypic scleractinian. Coral Reefs 5: 15-159

Dunlap WC, Chalker BE, Bandaranayake WM (1988) New sunscreening agents derived from tropical marine organisms of the Great Barrier Reef, Australia. In: Choat JH (ed) Proceedings of 20th international coral reef symposium, vol 3. Sixth Internatrional Coral Reef Executive Committee, Townsville, p 89-93

Dunlap WC, Chalker BE, Oliver JK (1986) Bathymetric adaptations of reef–building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J Exp Mar Biol Ecol 104: 239-248

Dunlap WC, Williams DMcB, Chalker BE, Banaszak AT (1989) Biochemical photoadaptation in vision; U.V.–absorbing pigments in fish eye tissues. Comp Biochem Physiol 93B: 601-607

Eberlein K, Leal MT, Hammer KD, Hickel W (1985) Dissolved organic substances during a Phaeocystis pouchetii bloom in the German Bight (North Sea). Mar Biol 89: 311-316

Eilertsen HC (1989) Phaeocystis pouchetii (Hariot) Lagerheim, a key species in Arctic marine ecosystems: Life history and physiology. Rapp P-v Réun Cons Perm Int Explor Mer 188: 131

Eilertsen HC, Schei B, Taasen JP (1981) Investigations on the plankton community of Balsfjorden, nothern Norway: The phytoplankton 1976-1978. Abundance, species composition, and succession. Sarsia 66: 129-141

Eilertsen HC, Taasen JP (1984) Investigations on the plankton community of Balsfjorden, Nothern Norway: The phytoplankton 1976-1978. Environmental factors, dynamics of growth, and primary production. Sarsia 69: 1-15

Eilertsen HC, Taasen JP, Weslawski JM (1989) Phytoplankton studies in the fjords of West Spitzbergen: physical environment and production in spring and summer. J Plankton Res 11: 1245-1260

Ekelund NGA (1990) Effects of UV-B radiation on growth and motility of four phytoplankton species. Physiol Plant 78: 590-594

El-Sayed SZ (1970) On the productivity of the Southern Ocean (Atlantic and Pacific sectors). In: Holdgate MW (ed) Antarctic ecology 1. Conrad, New York, p 118-153

El-Sayed SZ (1971) Observations on phytoplankton bloom in the Weddell Sea. In: Llano GA, Wallen IE (eds) Biology of the Antarctic seas IV. Antarctic research series, vol 17, American Geophysical Union, Washington, DC, p 301-312

El-Sayed SZ (1984) Productivity of the Antarctic waters. In: Holm-Hansen O, Bolis L, Giles R (eds) Marine phytoplankton productivity. Springer Verlag, New York, p 19-34

El-Sayed SZ (1988) Fragile life under the ozone hole. Natural History 10: 73-80

El-Sayed SZ, Biggs DC, Holm-Hansen O (1983) Phytoplankton standing crop, primary productivity, and near surface nitrogenous nutrient fields in the Ross Sea, Antarctica. Deep-Sea Res 30: 871-886

El-Sayed SZ, Fryxell GA (1993) Phytoplankton. In: Friedman EI (ed) Antarctic microbiology. Wiley-Liss, New York, p 65-122

El-Sayed SZ, Taguchi S (1981) Primary production and standing crop of phytoplankton along the ice-edge in the Weddell Sea. Deep-Sea Res 28: 1017-1032

El-Sayed SZ, Stephens FC, Bidigare RR, Ondrusek ME (1990) Effect of ultraviolet radiation on Antarctic marine phytoplankton. In: Kerry KR, Hempel G (eds) Antarctic ecosystems. Ecological change and conservation. Springer-Verlag, Berlin, p 379-385

El-Sayed SZ, Weber LH (1982) Spatial and temporal variations in phytoplankton biomass and primary productivity in the southwest Atlantic and the Scotia Sea. Polar Biol 1: 83-90

Eppley RW, Ried FMH, Strickland JDH (1970) Estimates of phytoplankton crop size, growth rate and primary production. In: Strickland JDH (ed) The ecology of the phytoplankton of La Jolla, California in the period April through September, 1967. Bull Scripps Inst Oceanogr 17: 33-42

Estep KW, Davis PG, Hargraves PE, Sieburth JMcN (1984) Chloroplast containing microflagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of Chrysochromulina (Prymnesiophyceae). Protistologica 20: 613-634

Estep KW, MacIntyre F (1989) Taxonomy, life cycle, distribution and dasmotrophy of Chrysochromulina: a theory accounting for scales, haptonema, muciferous bodies and toxicity. Mar Ecol Prog Ser 57: 11-21

Estep KW, Nejstgaard JC, Skjoldal HR, Rey F (1990) Predation of copepods upon natural populations of Phaeocystis pouchetii as a function of the physiological state of the prey. Mar Ecol Prog Ser 67: 235-249

Everitt DA, Thomas DP (1986) Observations of seasonal changes in diatoms at inshore localities near Davis Station, East Antarctica. Hydrobiologia 139: 3-12

Farman JC, Gardiner BG, Shanklin JD (1985) Large losses of total ozone in Antarctica reveal seasonal ClO_3/NO_3 interaction. Nature 315: 207-210

Fenchel T (1982) Ecology of heterotrophic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9: 35-42

Fenchel T (1987) Ecology of protozoa. Science Tech, Madison

Fernández E, Serret P, de Madariaga I, Harbour DS, Davies AG (1992) Photosynthetic carbon metabolism and biochemical composition of spring phytoplankton assemblages enclosed in microcosms: the diatom–Phaeocystis sp. succession. Mar Ecol Prog Ser 90: 89-102

Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. Mar Ecol Prog Ser 8: 37-52

Fischer AG (1965) Fossils, early life, and atmospheric history. Proc Natl Acad Sci USA 53: 1205-1215

Fischer G, Fuetterer D, Gersonde R, Honjo S, Ostermann D, Wefer G (1988) Seasonal variability of particle flux in the Weddell Sea and its relation to ice cover. Nature 335: 426-428

Foster P, Voltolina D, Spencer CP, Miller I, Beardall J (1983) A seasonal study of the distribution of surface state variables in Liverpool Bay. V. Summer. J Exp Mar Biol Ecol 73: 151-165

Frederick JE, Lubin D (1994) Solar ultraviolet radiation at Palmer Station, Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 43-52

Frederick JE, Snell HE (1988) Ultraviolet radiation levels during the antarctic spring. Science 241: 438-440

Fretter V, Montgomery MC (1968) The treatment of food by prosobranch veligers. J Mar Biol Ass UK 48: 499-520

Fryxell GA (1989) Marine phytoplankton at the Weddell Sea ice edge: seasonal changes at the specific level. Polar Biol 10: 1-18

Fryxell GA, Kendrick GA (1988) Austral spring microalgae across the Weddell Sea ice edge: spatial relationships found along a northward transect during AMEREZ 83. Deep-Sea Res 35: 1-20

Fryxell GA, Theriot EC, Buck KR (1984) Phytoplankton, ice algae, and choanoflagellates from AMERIEZ, the southern Atlantic and Indian Oceans. Antarct J US 19: 107-109

Fukuchi M (1980) Phytoplankton chlorophyll stocks in the Antarctic Ocean. J Oceanogr Soc Japan 36: 73-84

Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl Environ Microbiol 39: 1085-1095

Gabbot PA, Walker AJM (1971) Changes in the condition index and biochemical content of adult oyster (Ostrea edulis L.) maintained under hatchery conditions. J Cons Perm Int Explor Mer 34: 99-106

Gala WR, Giesy JP (1991) Effects of ultraviolet radiation on the primary production of natural phytoplankton assemblages in Lake Michigan. Ecotoxicol Environ Safety 22: 345-361

Garabétian F (1991) ^{14}C-glucose uptake and ^{14}C-CO_2 production in surface microlayer and surface-water samples: influence of UV and visible radiation. Mar Ecol Prog Ser 77: 21-26

Garrison DL (1991a) An overview of the abundance and role of protozooplankton in Antarctic waters. J Mar Syst 2: 317-331

Garrison DL (1991b) Antarctic sea ice biota. Amer Zool 31: 17-33

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

Garrison DL, Buck KR (1989a) Protozooplankton in the Weddell Sea, Antarctica: abundance and distribution in the ice-edge zone. Polar Biol 9: 341-351

Garrison DL, Buck KR (1989b) The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsula regions. Polar Biol 10: 211-219

Garrison DL, Buck KR, Fryxell GA (1987) Algal assemblages in the antarctic pack ice and in ice-edge plankton. J Phycol 23: 564-572

Garrison DL, Buck KR, Gowing MM (1991) Plankton assemblages in the ice edge zone of the Weddell Sea during the austral winter. J Mar Syst 2: 123-130

Garrison DL, Buck KR, Silver MW (1983) Studies of ice algal communities in the Weddell Sea. Antarct J US 18: 179-181

Garrison DL, Gowing MM (1992) Protozooplankton. In: Friedmann EI (ed) Antarctic microbiology. Wiley-Liss, New York, p 123-166

Garrison DL, Van Scoy K (1985) Wilkes Land Expedition 1985: Biological observations in the ice-edge zone. Antarct J US 20: 123-124

Gautier C, He G, Yang S (1994) Role of clouds and ozone on spectral ultraviolet-B radiation and biologically active UV dose over Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 93-110

Gersonde R (1986) Siliceous microorganisms in the sea ice and their record in sediments in the Southern Weddell Sea (Antarctica). In: Ricard M (ed) Proc 8th international diatom symposium. Paris, 1984, p 549-566

Gibson JAE, Garrick RC, Burton HR (1990) The annual cycle of bacterial numbers at an inshore site near the antarctic continent. Proc NIPR Symp Polar Biol 3: 16-22

Gibson JAE, Garrick RC, Burton HR, McTaggart AR (1989) Dimethylsulfide concentrations in the ocean close to the antarctic continent. Geomicrobiol J 6: 179-185

Gibson JAE, Garrick RC, Burton HR, McTaggart AR (1990) Dimethylsulfide and the alga Phaeocystis pouchetii in antarctic coastal waters. Mar Biol: 104 339-346

Gibson JAE, Swadling KM, Burton HR (1995) Acrylic acid and DMSP concentrations during an Antarctic phytoplankton bloom: how important is Phaeocystis? In: First

international symposium on DMSP and related sulfonium compounds (program and abstracts). University of South Alabama and Dauphin Island Sea Lab, p 21

Gibson JAE, Swadling KM, Burton HR (submitted) Interannual variation in dominant phytoplankton species and biomass near Davis Station, East Antarctica. Proc NIPR Symp Polar Biol

Gieskes WWC, van Bennekom AJ (1973) Unreliability of the ^{14}C method for estimating primary production in Dutch coastal waters. Limnol Oceanogr 18: 494-495

Gieskes WWC, Kraay G (1975) The phytoplankton spring bloom in Dutch coastal waters of the North Sea. Neth J Sea Res 9: 166-196

Gieskes WWC, Kraay GW (1977) Continuous plankton records: changes in the plankton of the North Sea and its eutrophic Southern Bight from 1948 to 1975. Neth J Sea Res 11: 334-364

Gieskes WWC, Kraay GW (1986) Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the flagellate Corymbellus aureus during the spring bloom in the open northern North Sea in 1983. Mar Biol 92: 45-52

Gieskes WWC, Kraay GW (1990) Transmission of ultraviolet light in the Weddell Sea: report of the first measurements made in the Antarctic. BIOMASS Newsl, College Station, Texas, 12: 12-14

Goes JI, Nobuhiko H, Taguchi S, Hama T (1994) Effect of UV-B radiation on the fatty acid composition of the marine phytoplankter Tetraselmis sp.: relationship to cellular pigments. Mar Ecol Prog Ser 114: 259-274

Gradinger RR, Baumann MEM (1991) Distribution of phytoplankton communities in relation to the large-scale hydrographic regime in the Fram Strait. Marine Biol 111: 311-321

Gran HH (1902) Das plankton des Norwegischen Nordmeers von biolischen Gesichtspunkten behandelt. Rep Norweg Fish Mar Inv 2: 1-222

Gran HH (1929) Investigation of the production of phytoplankton outside the Romsdalsfjord 1926-1927. Rapp P-v Réun Cons Perm Int Explor Mer 56: 1-112

Gran HH (1930) The spring growth of the phytoplankton at Møre in 1928-29 and at Lofoten in 1929 in relation to its limiting factors. Det Norske-Vidensk Akad Oslo, I. Mat Naturv Kl 5: 1-77

Granéli E, Granéli W, Rabbani MM, Daugbjerg N, Fransz G, Cuzin-Roudy J, Alder V A (1993) The influence of copepod and krill grazing on the species composition of phytoplankton communities from the Scotia-Weddell sea. Polar Biol 13: 201-213

Grant PT, Middleton C, Plack PA, Thomson RH (1985) The isolation of four aminocyclohexenimines (mycosporines) and a structurally related derivative of cyclohexane-1:3-dione (gadusol) from the brine shrimp Artemia. Comp Biochem Physiol (B) Comp Biochem 80: 755-759

Green JC (1976) Corymbellus aureus gen. et. sp. nov., a new colonial member of the Haptophyceae. J Mar Biol Ass UK 56: 31-38

Green RC (1962) Biosynthesis of dimethyl–β–propiothetin. J Biol Chem 237: 2251-2254

Grimm N, Weisse T (1985) Die temperaturabhängigkeit des wachstums von Phaeocystis pouchetii (Haptophyceae) in batchkulturen. Helgoländer Wiss Meeresunters 39: 201-211

Grontved J (1960) Planktological contribution IV. Taxonomic and productional investigations in shallow coastal waters. Meddr Kommn Danm Fisk - og Havunder 3: 1-17

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

Grossi SM, Sullivan CW (1985) Sea ice microbial communities. V. The vertical zonation of diatoms in an Antarctic fast ice community. J Phycol 21: 401-409

Guillard RRL, Hellebust JA (1971) Growth and the production of extracellular substances by two strains of Phaeocystis pouchetii. J Phycol 7: 330-338

Guillard RRL, Ryther JH (1962) Studies of the marine plankton diatoms 1. Cyclotella nana Hustedt and Detonula confervaceae (Cleve) Gran. Can J Microbiol 8: 229-239

Gunkel W (1982) Mikrobiologisch-chemische untersuchungen an meeresschaum. Jahresber. 1981. Biol Anst Helgoländ, p 49-50

Häder D-P (1986) Effects of solar and artificial UV radiation on motility and phototaxis in the flagellate Euglena gracilis. Photochem Photobiol 44, 651-656

Häder D-P (1987) Effects of UV-B radiation on photomovement in the desmid, Cosmarium cucumis. Photochem Photobiol 46 121-126

Häder D-P (1988) Ecological consequences of photomovement in microorganisms. Photochem Photobiol 1 (B): 385-414

Häder D-P (1993) Effect of enhanced solar ultraviolet radiation on aquatic ecosystems. In: Tevini M (ed) UV-B radiation and ozone depletion; effects on humans, animals, plants, microorganisms and materials. Lewis Publishers, Boca Raton, p 155-192

Häder D-P, Worrest RC (1991) Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. Photochem Photobiol 53: 717-725

Haeckel E (1890) Plankton-stidien. Vergleichende Untersuchungen über die Bedeutung und Zusammensetzung der pelagischen fauna and flora. Jena Ztschr Naturwiss 25: 232-236

Hallegraeff GM (1983) Scale-bearing and loricate nanoplankton from the East Australian current. Bot Mar 26: 493-515

Hannan PJ, Swinnerton JW, Lamontagne RA, Patouillet C (1980) Effect of UV-B on algal growth rate and trace gas production. In: Eaton JG (ed) Aquatic toxicology. American society for testing and materials, p 177-190

Hansen B, Tande KS, Berggreen UC (1990) On the trophic fate of Phaeocystis pouchetii (Hariot). III. Functional responses in grazing demonstrated on juvenile stages of Calanus finmarchicus (Copepoda) fed diatoms and Phaeocystis. J Plankton Res 12: 1173-1187

Hansen B, Verity P, Falkenhaug T, Tande KS, Norrbin F (1994) On the trophic fate of Phaeocystis pouchetii (Harriot). V. Trophic relationships between Phaeocystis and

zooplankton: an assessment of methods and size dependence. J Plankton Res 16: 487-511

Hansen FC (1992) Zooplankton-grazing an Phaeocystis mit besonderer Berückstichtigung der calanoiden copepod. Ber Inst Meeresk Keil 229: 1-37

Hansen FC, Reckermann M, Klein Breteler WCM, Reigman R (1993) Phaeocystis blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Mar Ecol Prog Ser 102: 51-57

Hansen FC, van Boekel WHM (1991) Grazing pressure of the calanoid copepod Temora longicornis on a Phaeocystis dominated spring bloom in a Dutch tidal inlet. Mar Ecol Prog Ser 78: 123-129

Hanson RB, Lowery HK, Shafer D, Sorocco R, Pope DH (1983a) Microbes in Antarctic water of the Drake Passage: Vertical patterns of substrate uptake, productivity and biomass in January 1980. Polar Biol 2: 179-188

Hanson RB, Shafer D, Ryan T, Pope DH, Lowery HK (1983b) Bacterioplankton in Antarctic Ocean waters during late austral winter: abundance, frequency of dividing cells, and estimates of production. Appl Environ Microbiol 45: 1622-1632

Hardy JT (1982) The sea surface microlayer: biology, chemistry and anthropogenic enrichment. Prog Oceanogr 11: 307-328

Hardy J, Gucinski H (1989) Stratospheric ozone depletion: implications for marine ecosystems. Oceanography 2: 18-21

Harm W (1980) Biological effects of ultraviolet radiation. Cambridge University Press, Cambridge

Hart TJ (1942) Phytoplankton periodicity in Antarctic surface waters. Disc Rep 21: 261-356

Hasle GR (1969) An analysis of the phytoplankton of the Pacific Southern Ocean: abundance composition and distribution during the Brategg Expedition 1947-1948. Hvalråd Skr 52: 6-128

Hasle GR, Medlin LK (1990) Family Bacillariaceae. In: Medlin LK, Priddle J (eds) Polar marine diatoms. British Antarctic Survey, UK, p 169-176

Hatakeyama S, Izumi K, Akimoto H (1985) Yield of SO_2 and formation of aerosol in the photo-oxidation of DMS under atmospheric conditions. Atmosph Environ 19: 135-141

Hedgepeth JW (1977) The Antarctic marine ecosystem. In: Llando JL (ed) Adaptations within the Antarctic ecosystem. Proceedings of the third SCAR symposium on Antarctic biology. Smithsonian Institute, Washington, DC, p 214-236

Helbling EW, Marguet ER, Villafañe V, Holm-Hansen O (1995) Bacterioplankton viability in Antarctic waters as affected by solar ultraviolet radiation. Mar Ecol Prog Ser 126: 293-298

Helbling W, Villafañe V, Ferrario M, Holm-Hansen O (1992) Impact of natural ultraviolet radiation on specific marine phytoplankton species. Mar Ecol Prog Ser 80: 89-100

Helbling EW, Villafañe V, Holm-Hansen, O (1994) Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 207-228

Hemmingsen EA, Douglas EL (1970) Ultraviolet radiation thresholds for Antarctic and temperate-zone animals. Comp Biochem Physiol 32: 593-600

Hempel G (1985) Antarctic marine food webs. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Fourth SCAR symposium on Antarctic biology. Springer-Verlag, Berlin, p 266-271

Hentschel E (1936) Die biologischenmethoden und das biologische beobachtungsmaterial der Meteor expedition. Wissenschaften ergebnis Deutsch Antarctic expedition Meteor 1925-27 10: 1-274

Henderson JR, Olsen RE, Eilertsen HC (1991) Lipid composition of phytoplankton from the Barents Sea and environmental influences on the distribution pattern of carbon among photosynthetic end products. Polar Res 10: 229-237

Herndl GJ, Müller-Niklas G, Frick J (1993) Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. Nature 361:717-719

Hewes CD, Holm-Hansen O, Sakshaug E (1985) Alternate carbon pathways at lower trophic levels in the antarctic food web. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs (Proceedings of the 4th SCAR Symposium on Antarctic Biology). Springer-Verlag, Berlin, p 277-283

Heywood RB, Priddle J (1987) Retention of phytoplankton by an eddy. Cstl Shelf Res 7: 937-955

Hickel W (1984) Seston in the Wadden Sea of Sylt (German Bight, North Sea). Neth Inst Sea Res Publ Ser 10: 113-131

Higginbottom IR, Hosie GW (1989) Biomass and population structure of a large aggregation of krill near Prydz Bay, Antarctica. Mar Ecol Prog Ser 58: 197-203

Hobson LA, Hartley FA (1983) Ultraviolet irradiance and primary production in a Vancouver Island fjord, British Columbia, Canada. J Plankton Res 5: 325-331

Hoffman DJ (1989) Direct ozone depletion in springtime Antarctic lower stratospheric clouds. Nature 326: 447-449

Holligan PM (1987) The physical environment of exceptional phytoplankton blooms in the northeast Atlantic. Rapp P-v Réun Cons Perm Int Explor Mer 187: 9-18

Hollowday ED (1949) A preliminary report on the Plymouth marine and brackish-water rotifer. J Mar Biol Ass UK 28: 239-254

Holm-Hansen O (1985) Nutrient cycles in Antarctic marine ecosystems. In: Siegfried WR, Cody PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer-Verlag, Berlin, p 6-10

Holm-Hansen O (1990) Effects of ultraviolet-B and ultraviolet-A on photosynthetic rates on Antarctic phytoplankton. Antarct J US 25: 177-178

Holm-Hansen O, El-Sayed SZ, Franceochini GA, Cuhel RL (1977) Primary production and the factors controlling phytoplankton growth in the Southern Ocean. In: Llando GL (ed) Adaptations within the Antarctic ecosystem. Proceedings of the third SCAR symposium on Antarctic biology. Smithsonian Institute, Washington, DC, p 3-10

Holm-Hansen O, Helbling EW (1993) Polythene bags and solar ultraviolet radiation. Science 259: 534

Holm-Hansen O, Helbling EW, Lubin D (1993) Ultraviolet radiation in Antarctica: inhibition of primary production. Photochem Photobiol 59: 567-570

Holm-Hansen O, Mitchell BG (1990) Effect of solar UV radiation on photosynthetic rates of Antarctic marine phytoplankton. Eos 71: 138

Holm-Hansen O, Mitchell BG, Hewes CD, Karl DM (1989a) Phytoplankton blooms in the vicinity of Palmer Station, Antarctica. Polar Biol 10: 49-57

Holm-Hansen O, Mitchell BG, Vernet M (1989b) Ultraviolet radiation in antarctic waters: effects on rates of primary production. Antarct J US 24: 177-178

Hooker JD (1847) Diatomaceae. The botany of the Antarctic voyage of H.M. Discovery ships Erebus and Terror, years 1839–1843, vol 56. London

Horner R (1990) Ice-associated ecosystems. In: Medlin LD, Priddle J (eds) Polar marine diatoms. British Antarctic Survey, Cambridge, p 9-18

Horner RA, Syvertsen EE, Thomas DP, Lange C (1988) Proposed terminology and reporting units for sea ice algal assemblages. Polar Biol 8: 249-253

Hoshiai T (1977) Seasonal changes of ice communities in the sea-ice near Syowa station, Antarctica. In: Dunbar MJ (ed) Polar Oceans. Arctic Institute of North America, Calgary, p 307-317

Hoshiai T (1981) The plant pigments, chlorinity and pH distribution in the sea ice of the Syowa Station area in 1970. Natl Inst Polar Res, Data Reports 67: 1-42

Houghton RA, Woodwell GM (1989) Global climatic change. Sci Amer 260: 36-44

Hughes J, McCully ME 1975 The use of an optical brightener in the study of plant structure. Stain Tech 50: 319-329

Hunter JR, Kaupp SE, Taylor JH (1981) Effects of solar and artificial ultraviolet-B radiation on larval northern anchovy, Engraulis mordax. Photochem Photobiol 34: 477-486

Huntley M, Tande K, Eilertsen HC (1987) On the trophic fate of Phaeocystis pouchetii (Hariot). II. Grazing rates of Calanus hyperboreus feeding on diatoms and different size categories of P. pouchetii. J Exp Mar Biol Ecol 110: 197-212

IPCC (1994) Radiative forcing of climate change: summary for policy makers. The 1994 report of the scientific assessment of IPCC (Intergovernmental Panel on Climate Change), UNEP, WMO

Ito S, Hirata Y (1977) Isolation and structure of mycosporine from the zoanthid Palythoa tuberculosa. Tetrahedron Lett 28: 325-338

Iverson RL, Whitledge TE, Goering JJ (1979) Chlorophyll and nitrate fine structure in the southeastern Bering Sea shelf break front. Nature 281: 664-666

Jacka TH 1983 A computer data base for Antarctic sea ice extent. ANARE Research Notes, No 13, Australian Antarctic Division, Hobart

Jacques G (1983) Some ecophysiological aspects of the Antarctic phytoplankton. Polar Biol 2: 27-33

Jacques G, Panouse M (1991) Biomass and composition of size fractionated phytoplankton in the Weddell–Scotia confluence area. Polar Biol 11: 315-328

Jahnke J (1989) The light and temperature dependence of growth rate and elemental composition of Phaeocystis globosa Scherffel and P. pouchetii (Har.) Lagerh. in batch cultures. Neth J Sea Res 23: 15-21

Jahnke J, Baumann MEM (1986) Die marine planktonalge Phaeocystis globosa; Eine massenform unserer küstengewässer. Mikrokosmos 75: 357-359

Jahnke J, Baumann MEM (1987) Differenciation between Phaeocystis pouchetii (Har.) Lagerheim and Phaeocystis globosa Scherffel. I. Colony shapes and temperature tolerances. Hydrobiol Bull 21: 141-147

Jeffrey SW (1981) Phytoplankton ecology - with particular reference to the Australian region. In: Clayton MN, King RJ (eds) Marine botany: an Australian perspective. Longman Cheshire, Melbourne, p 241-292

Jeffrey SW, Hallegraeff GM (1987) Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar Ecol Prog Ser 35: 293-304

Jeffrey SW, Wright SW (1994) Photosynthetic pigments in the Haptophyta. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. The Systematic Association Special Vol 51, Clarendon Press, Oxford, p 111-132

Jennings JC, Gordon LI, Nelson DM (1984) Nutrient depletion indicates high primary productivity in the Weddell Sea. Nature 399: 51-54

Jerlov NG (1950) Ultra-violet radiation in the sea. Nature 166: 111-112

Jialal J, Norkus EP, Cristol L, Grundy SM (1991) β–carotene inhibits the oxidative modification of low-density lipoprotein. Biochem Biophys Acta 1086: 134-138

Jitts HR, Morel A, Saijo Y (1976) The relation of oceanic primary production to available photosynthetic irradiance. Aust J Mar Freshw Res 27: 441-454

Joint I, Pomroy A (1993) Phytoplankton biomass and production in the southern North Sea. Mar Ecol Prog Ser 99: 169-182

Joint IR, Pomroy AJ (1981) Primary production in a turbid estuary. Estuar Cstl Shelf Sci 13: 303-316

Joiris C, Billen G, Lancelot C, Daro MH, Mommaerts J P, Bertels A, Bossicart M, Nijs J, Hecq JH (1982) A budget of carbon cycling in the Belgian coastal zone: relative role of zooplankton, bacterioplankton and benthos in the utilisation of primary production. Neth J Sea Res 16: 260-275

Jokiel PL, York RH Jr (1984) Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnol Oceanogr 29: 192-199

Jones LW, Kok B (1966) Photoinhibition of chloroplast reactions. I. Kinetics and action spectrum. Plant Physiol 41: 1037-1043

Jones AE, Shanklin JD (1995) Continued decline of total ozone over Halley, Antarctica, since 1985. Nature 376: 409-411

Jones M, Spencer PC (1970) The phytoplankton of the Menai Straits. J Cons Perm Int Explor Mer 33: 169-180

Jones PGW, Haq SM (1963) The distribution of Phaeocystis in the eastern Irish Sea. J Cons Perm Int Explor Mer 28: 8-20

Kang S-H, Fryxell GA (1991) Most abundant diatoms species in the water column from five ODP leg 119 drill sites in Prydz Bay, Antarctica: distributional patterns. In: Barron J, Larsen B (eds) Proceedings of the ocean drilling program: Scientific Results. 119: 645-666

Kang S-H, Fryxell GA (1992) Fragilariopsis cylindrus (Grunow) Krieger: the most abundant diatom in the water column assemblages in Antarctic marginal ice-edge zones. Polar Biol 12: 609-627

Kang S-H, Fryxell GA (1993) Phytoplankton in the Weddell Sea, Antarctica: composition, abundance and distribution in the water-column assemblages of the marginal ice-edge zone during austral autumn. Mar Biol 116: 335-348

Kang S-H, Lee SH (1995) Antarctic phytoplankton assemblage in the western Bransfield Strait region, February 1993: composition, biomass, and mesoscale distributions. Mar Ecol Prog Ser 129: 253-267

Karentz D (1988) DNA repair mechanisms in Antarctic marine microorganisms. Antarct J US 23: 114-115

Karentz D (1989) Report on studies related to the ecological implications of ozone depletion on the Antarctic environment. Antarct J US 24: 175-176

Karentz D (1990) Ecological considerations of the Antarctic ozone hole in the marine environment. In: Blough NV, Zepp RG (eds) Effects of solar ultraviolet radiation on biogeochemical dynamics in aquatic environments. Woods Hole Oceanographic Institution Tech Rept, WHOI-90-09, p 137 - 140

Karentz D (1991) Ecological considerations of Antarctic ozone depletion. Antarct Sci 3: 3-11

Karentz D (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 93-110

Karentz D, Cleaver JE, Mitchell DL (1991a) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. J Phycol 27: 326-341

Karentz D, Cleaver JE, Mitchell DL (1991c) DNA damage in the Antarctic. Nature 350: 28

Karent D, Lutze LH (1990) Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol Oceanogr 35: 549-561

Karentz D, McEuen FS, Land MC, Dunlap WC (1991b) Survey of mycosporine–like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108: 157-166

Karentz D, Spero HJ (1995) Response of natural Phaeocystis populations to ambient fluctuations in UVB radiation caused by Antarctic ozone depletion. J Plankton Res 17: 1771-1789

Karsten U, Kück K, Vogt C, Kirst GO (1995) DMSP production in phototrophic organisms and its physiological function as cryoprotectant. In: First international symposium on DMSP and related sulfonium compounds (program and abstracts). University of South Alabama and Dauphin Island Sea Lab, p 31

Kashkin NI (1963) Material in the ecology of Phaeocystis pouchetii (Hariot) Lagerheim, 1893 (Chrysophyceae). II. Habitat and specifications of biogeographical characteristics. Okeanologiya 3: 697-705

Kawaguti S (1969) Effect of green fluorescent pigments on the productivity of reef corals (abstract). Micronesica 5: 313

Kayser A (1970) Experimental-ecological investigations on Phaeocystis pouchetii (Haptophyceae): cultivation and waste water test. Helgolander wiss Meeresunters 20: 195-212

Keller MD, Bellows WK, Guillard RRL (1989) Dimethyl sulfide production in marine phytoplankton. In: Saltzmann ES, Cooper WJ (eds) Biogenic sulfur in the environment. ACS Symposium Series 393, American Chemical Society, Washington DC, p 167-182

Kieber DJ, McDaniel J, Mopper K (1989) Photochemical source of biological substrates in sea water: implications for carbon cycling. Nature 341: 637-639

Kiene RP, Bates TS (1990) Biological removal of dimethyl sulfide from sea water. Nature 345: 702-705

Kirk JTO (1983) Light and photosynthesis in aquatic ecosystems. Cambridge University Press, Cambridge

Kitchen JC, Zaneveld JR (1993) A three layered optical model of the optical properties of phytoplankton. Limnol Oceanogr 37: 1680-1690

Kivi K, Kuosa H (1994) Late winter microbial communities in the western Weddell Sea (Antarctica). Polar Biol 14: 389-399

Knox GA (1988) Primary production and consumption in McMurdo Sound, Antarctica. In: Kerry KR, Hempel G (eds) Antarctic ecosystems. Ecological change and conservation. Springer-Verlag, Berlin, p 115-128

Kobayashi J, Nakamura H, Hirata Y (1981) Isolation and structure of a UV–absorbing substance 337 from the ascidian Halocynthia roretzi. Tetrahedron Lett 22: 3001-3002

Kogure K, Fukami K, Simidu U, Taga N (1986) Abundance and production of bacterioplankton in the Antarctic. Mem Nat Inst Polar Res Spec Issue 40: 414-422

Kornmann VP (1955) Beobachtungen an Phaeocystis-Kulturen. Helgolander Wiss Meeresunters 5: 218-233

Kottmeier ST, Sullivan, CW (1990) Bacterial biomass and production in pack ice of Antarctic marginal ice edge zones. Deep-Sea Res 37: 1311-1330

Krebs WM (1983) Ecology of neritic marine diatoms, Arthur Harbor, Antarctica. Micropaleontology 29: 267-297

Künne C (1952) Untersuchungen über das grossplankton in der Deutschen Bucht und im Nordsylter Wattenmeer. Helgoländer Wiss Meeresunters 4: 1-54

Kwint RLJ, Kramer KJM (1995) Dimethylsulphide production by plankton communities. Mar Ecol Prog Ser 121: 227-237

Laanbroek HJ, Verplanke JC, de Visscher PRM, de Vuyst R (1985) Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, the Netherlands. Mar Ecol Prog Ser 25: 1-11

Lagerheim G (1893) Phaeocystis, nov. gen., grundadt på Tetraspora Poucheti Har. Bot Notiser 1: 32-33.

Lagerheim G (1896) Ueber Phaeocystis pouchetii (Hariot) Lagerheim, eine plankton-flagellate. Öefvers Kongl Svenska Vet Akad Forhandl 53: 277-288

Lampert W (1978) Release of DOC by grazing zooplankton. Limnol Oceanogr 23: 831-834

Lancelot C (1983) Factors affecting phytoplankton extracellular release in the Southern Bight of the North Sea. Mar Ecol Prog Ser 12: 115-121

Lancelot C (1984a) Extracellular release of small and large molecules by phytoplankton in the southern bight of the North Sea. Estuar Cstl Shelf Sci 18: 65-77

Lancelot C (1984b) Metabolic changes in Phaeocystis pouchetii (Hariot) Lagerheim during the spring bloom in Belgian coastal waters. Estuar Cstl Shelf Sci 18: 593-600

Lancelot C, Billen G (1984) Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. Limnol Oceanogr 29: 721-730

Lancelot C, Billen G, Sournia A, Weisse T, Colijn F, Veldhuis MJW, Davies A, Wassmann P (1987) Phaeocystis blooms and nutrient enrichment in the continental coastal zones of the North Sea. Ambio 16: 38-46

Lancelot C, Mathot S (1985) Biochemical fractionation of primary production of phytoplankton in Belgian coastal waters during short– and longterm incubation with ^{14}C-bicarbonate. II. Phaeocystis pouchetii colonial population. Mar Biol 86: 227-232

Lancelot C, Mathot S (1987) Dynamics of a Phaeocystis-dominated spring bloom in Belgian coastal waters. I. Phytoplanktonic activity and related parameters. Mar Ecol Prog Ser 37: 239-248

Lancelot C, Mathot S, Owens NJP (1986) Modelling protein synthesis, a step to an accurate estimate of net primary production: Phaeocystis pouchetii colonies in Belgian coastal waters. Mar Ecol Prog Ser 32: 193-202

Lancelot C, Rousseau V (1994) Ecology of Phaeocystis: the key role of colony forms. In: Green J C, Leadbeater BSC (eds) The haptophyte algae. The Systematic Association Special Vol 51, Clarendon Press, Oxford, p 229-245

Lancelot C, Wassmann P, Barth H (1994) Ecology of Phaeocystis–dominated ecosystems. J Mar Syst 5: 1-4

Lebour MV (1922) The food of plankton organisms. J Mar Biol Ass UK 12: 644-677

Ledyard K, Dacey JWH (1990) Production of DMS from DMSP by a marine bacterium (abstract). Eos 71: 104

Legendre L (1990) The significance of microalgal blooms for fisheries and for the export of particulate organic carbon in oceans. J Plankton Res 12: 681-699

Leoblich AR III, Smith VE (1968) Chloroplast pigments of the marine dinoflagellate Gymnodinium resplendens. Lipids 3: 3-15

Lessard RM, Rivkin RB (1986) Nutrition of microzooplankton and macrozooplankton from McMurdo Sound. Antarct J US 21: 187-188

Lesser MP, Cullen JJ, Neale PJ (1994) Carbon uptake in a marine diatom during acute exposure to ultraviolet-B radiation: Relative importance of damage and repair. J Phycol 30: 183-192

Letelier RM, Karl DM (1989) Phycoerythrin–containing cyanobacteria in surface waters of the Drake Passage during February 1987. Antarct J US 24: 185-188

Lipski M (1987) Variations of physical conditions, nutrients and chlorophyll a contents in Admiralty Bay (King George Island, South Shetland Islands, 1979). Polar Res 8: 307-332

Liss PS, Malin G, Turner SM, Holligan PM (1994) Dimethyl sulphide and Phaeocystis: a review. J Mar Syst 5: 41-55

Lizotte MP, Sullivan CW (1991) Rates of photoadaptation in sea ice diatoms from McMurdo Sound, Antarctica. J Phycol 27: 367-373

Lorenzen CJ (1967) Determination of chlorophyll a and phaeopigments: spectrophotometric equations. Limnol Oceanogr 12: 343-347

Lorenzen CJ (1979) Ultraviolet radiation and phytoplankton photosynthesis. Limnol Oceanogr 24: 1117-1120

Lovelock JE, Maggs RJ, Rasmussen RA (1972) Atmospheric dimethyl sulphide and the natural sulphur cycle. Nature 237: 418-420

Lubbers GW, Gieskes WWC, del Castilho P, Salomons W, Bril J (1990) Manganese accumulation in the high pH microenvironment of Phaeocystis sp. (Haptophyceae) colonies from the North Sea. Mar Ecol Prog Ser 59: 285-293

Lubin D, Frederick JE, Booth CR, Lucas T, Neuschuler D (1989) Measurements of enhanced springtime ultraviolet radiation at Palmer Station Antarctica. Geophys Res Lett 16: 783-785

Lubin D, Jensen EH (1995) Effects of clouds and stratospheric ozone depletion on ultraviolet radiation trends. Nature 377: 710-713

Lucas CE (1940) Ecological investigations with the continuous plankton recorder: the phytoplankton in the southern North Sea, 1932-1937. Hull Bull Mar Ecol 1: 73-170

Lutter S, Taasen JP, Hopkins CCE, Smetacek V (1989) Phytoplankton dynamics and sedimentation processes during spring and summer in Balsfjord, Northern Norway. Polar Biol 10: 113-124

Madronich S (1995) The radiation equation. Nature 377: 682-683

Malej A, Harris RP (1993) Inhibition of copepod grazing by diatom exudates: a factor in the development of mucus aggregates? Mar Ecol Prog Ser 96: 33-42

Malin G, Liss PS, Turner SM (1994) Dimethyl sulfide: production and atmospheric consequences. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. The Systematic Association Special Vol 51, Clarendon Press, Oxford, p 303-320

Marchant HJ (1982) Snow algae from the Australian Snowy Mountains. Phycologia 21: 178-184

Marchant HJ (1985) Choanoflagellates in the Antarctic marine food chain. In: Siegfried WR, Cody PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer, Berlin, p 271-276

Marchant HJ (1990) Grazing rate and particle size selection by the choanoflagellate Diaphanoeca grandis from the sea-ice of Lagoon Saroma-ko, Hokkaido. Proc NIPR Symp Polar Biol 3: 1-7

Marchant HJ (1993) Antarctic marine nanoplankton. Curr Topics Bot Res 1: 189-201

Marchant HJ (1994) Biological impacts of seasonal ozone depletion. In: Hempel G (ed) Antarctic science. Springer-Verlag, Berlin, p 95-109

Marchant HJ, Davidson AT (1991) Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. In: Weller G, Wilson CL, Severin BAB (eds) Proceedings of the International Conference on the Role of Polar Regions in Global Change. Geophysical Institute, Fairbanks, p 397-400

Marchant HJ, Davidson AT, Kelly GJ (1991) UV-B protecting pigments in the marine alga Phaeocystis pouchetii from Antarctica. Mar Biol 109: 391-395

Marchant HJ, Davidson AT, Wright SW (1987) The distribution and abundance of chroococcoid cyanobacteria in the Southern Ocean. Proc NIPR Symp Polar Biol 1:1-9

Marchant HJ, McEldowney A (1987) Nanoplanktonic siliceous cysts from Antarctica are aglae. Mar Biol 92: 53-57

Marchant HJ, McMinn A, Scott F (in prep) Antarctic phytroplankton atlas.

Marchant HJ, Murphey E (1993) Interactions at the base of the Antarctic food web. In: El-Sayed SZ (ed) Southern Ocean ecology; the BIOMASS perspective. Cambridge University Press, Cambridge p 267-285

Marchant HJ, Nash GV (1986) Electron microscopy of gut contents and faeces of Euphausia superba Dana. Mem Natl Inst Polar Res, Spec Issue 40: 167-177

Marchant HJ, Perrin RA (1990) Seasonal variation in abundance and species composition of choanoflagellates (Acanthoecideae) at Antarctic coastal sites. Polar Biol 10: 499-505

Marchant HJ, Scott FJ (1993) Uptake of sub-micrometre particles and dissolved organic material by Antarctic choanoflagellates. Mar Ecol Prog Ser 92: 59-64

Marchant HJ, Thomsen HA (1994) Haptophytes in polar waters. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. The Systematic Association Special Vol 51, Clarendon Press, Oxford, p 209-228

Margalef R (1978) The phytoplankton communities in upwelling areas. The example of NW Africa. Oecologia Aquatica 3: 97-132

Marr J (1962) The natural history and geography of Antarctic krill (Euphausia superba Dana). Disc Rep 32: 33-464

Marschall HP (1988) The overwintering strategy of Antarctic krill under the pack ice of the Weddell Sea. Polar Biol 9: 129-135

Marshall SM, Orr AP (1962) Carbohydrate as a measurement of phytoplankton. J Mar Biol Ass UK 42: 511-519

Martens P (1980) Beitrage zum mesozooplankton des Nordsylter Wattenmeeres. Helgoländer Wiss Meeresunters 34: 41-53

Martens P (1981) On the Acartia species in the northern Wadden Sea of Sylt. Kieler Meeresforsch Sonderh 5: 153-163

Martin JH, Gordon RM, Fitzwater SE (1990) Iron in Antarctic waters. Nature 345: 156-158

Maske H (1984) Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake. J Plankton Res 6: 351-357

Mathot S, Becquevort S, Lancelot C (1991) Microbial communities from the sea ice and adjacent water column at the time of ice melting in the northwestern part of the Weddell Sea. Polar Res 10: 267-277

Matrai PA, Keller MD (1994) Total organic sulfur and dimethylsulfoniopropionate in marine phytoplankton: intracellular variations. Mar Biol 119: 61-68

Matrai PA, Vernet M, Hood R, Jennings A, Brody E, Saemundsdóttir S (1995) Light-dependence of carbon and sulfur production by polar clones of the genus Phaeocystis. Mar Biol 124: 157-167

McConville MJ, Wetherbee R (1983) The bottom-ice microalgal community from annual ice in the inshore waters of East Antarctica. J Phycol 19: 431-439

McFadden GI, Moestrup O, Wetherbee R (1982) Pyramimonas gelidicola sp. nov. (Prasinophyceae), a new species isolated from Antarctic sea ice. Phycologia 21: 431-439

McKenzie D (1995) Ozone future is up in the air. New Scientist Dec Issue: 14-15

McMinn A, Heijnis H, Hodgson D (1994) Minimal effects of UVB radiation on Antarctic diatoms over the past 20 years. Nature 370: 547-549

McMinn A, Hodgson D (1993) Summer succession in Ellis Fjord, eastern Antarctica. J Plankton Res 15: 925-938

Maykut GA (1985) The ice environment. In: Horner RA (ed) Sea ice biota. CRC Press, Boca Raton, Florida, p 21-82

Medlin LK, Lange M, Baumann MEM (1994) Genetic differenciation among three colony-forming species of Phaeocystis: further evidence for the origin of the Prymnesiophyta. Phycologia 33: 199-212

Meyer MA, El-Sayed SZ (1983) Grazing of Euphausia superba Dana on natural phytoplankton populations. Polar Biol 1: 193-197

Miller DGM, Hampton I (1989) Biology and ecology of Antarctic krill (Euphausia superba Dana). BIOMASS Scientific Series 9, p 1-166

Mitchell BG, Holm-Hansen O (1991) Observations and modelling of the Antarctic phytoplankton crop in relation to mixing depth. Deep-Sea Res 38: 981-1007

Mitchell BG, Vernet M, Holm-Hansen O (1989) Ultraviolet light attenuation in Antarctic waters in relation to particulate absorption and photosynthesis. Antarct J US 24: 179-181

Mitchell DL, Karentz D (1990) Molecular and biological responses of antarctic phytoplankton to ultraviolet radiation. Antarct J US 25: 174-175

Moestrup Ø (1979) Identification by electron microscopy of marine nanoplankton from New Zealand, including the description of four new species. NZ J Bot 17: 61-95

Moestrup Ø (1994) Economic aspects: 'blooms', nuisance species, and toxins. In: Green JC, Leadbeater BSC (eds) The haptophyte algae. The Systematic Association Special Vol 51, Clarendon Press, Oxford, p 265-285

Moisan TA, Fryxell GA (1993) The distribution of Antarctic diatoms in the Weddell Sea during austral winter. Botanica Marina 36: 489-497

Mommaerts JP (1973) The relative importance of nanoplankton in the North Sea primary production. Br Phycol J 8: 13-20

Montzka SA, Butler JH, Myers RC, Thompson TM, Swanson TH, Clarke AD, Lock LT, Elkins JW (1996) Decline in the tropospheric abundance of halogen from halocarbons: implications for stratospheric ozone depletion. Science 272: 1318-1322

Moore AL, Joy A, Tom R, Gust D, Moore TA, Bensasson RV, Land EJ (1982) Photoprotection by carotenoids during photosynthesis: motional dependence of intramolecular energy transfer. Science 216: 982-984

Mopper K, Zhou X, Kieber RJ, Kieber DJ, Sikorski RJ, Jones RD (1991) Photochemical degradation of dissolved organic carbon and its impact on the oceanic carbon cycle. Nature 353: 60-62

Morel FMM, Reuter JG, Anderson DM, Guillard RRL (1979) Aquil: a chemical defined phytoplankton culture medium for trace metal studies. J Phycol 15: 135-141

Morris AW (1971) Trace metal variations in sea water of Menai Straits caused by a bloom of Phaeocystis. Nature 233: 427-428

Morris I, Glover HE, Yentsch CS (1974) Products of photosynthesis by marine phytoplankton: the effects of environmental factors on the relative rates of protein synthesis. Mar Biol 27: 1-9

Mortan-Bertrand A (1988) Photosynthetic metabolism of an Antarctic diatom and its physiological responses to fluctuations in light. Polar Biol 9: 53-60

Mortan-Bertrand A (1989) Effects of light fluctuations on the growth and productivity of Antarctic diatoms in culture. Polar Biol 9: 245-252

Müller-Niklas G, Heissenberger A, Puskari´c S, Herndl G (1995) Ultraviolet-B radiation and bacterial metabolism in coastal waters. Aquatic Microb Ecol 9: 111-116

Mura PM, Satta MP, Agusti S (1995) Water-mass influences on summer Antarctic phytoplankton biomass and community structure. Polar Biol 15: 15-20

Murray N, Gabric AJ, Stone L, Realini G, Kohl M (1992) The production and cycling of dimethylsulfide in marine surface waters - a simulation approach. Fresenius Envir Bull 1: 274-279

Murray AG, Jackson GA (1993) Viral dynamics: a model of the interaction of ultraviolet light and mixing processes on virus survival in seawater. Mar Ecol Prog Ser 102: 105-114

Nakamura HJ, Kobayashi J, Hirata Y (1981) Isolation of a 330 nm UV-absorbing substance, asterina-330 from the starfish Asterina pectinifera. Chem Lett 1981: 1433-1414

Nakamura H, Kobayashi J, Hirata Y (1982) Separation of mycosporine–like amino acids in marine organisms using reverse-phase high-performance liquid chromatography. J Chromatogr 250: 113-118

Neale PJ, Lesser MP, Cullen JJ (1994) Effects of ultraviolet radiation on photosynthesis of phytoplankton in the vacinity of McMurdo Station, Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 125-142

Nelson DM, Gordon LI (1982) Production and pelagic dissolution of biogenic silica in the Southern Ocean. Geochim Cosmochim Acta 46: 491-501

Nelson DM, Gordon LI, Smith WO Jr (1985) Phytoplankton dynamics of the marginal ice zone of the Weddell Sea, November and December 1983. Antarct J US 19: 105-107

Nelson DM, Treguer P (1992) Role of silicon as a limiting nutrient to Antarctic diatoms: evidence from kinetic studies in the Ross Sea ice-edge zone. Mar Ecol Prog Ser 80: 255-264

Neori A, Holm-Hansen O (1982) Effect of temperature on the rate of photosynthesis in Antarctic phytoplankton. Polar Biol 1: 33-38

Nicholls AG (1935) The larval stages of Longipedia coronata Claus, L. scotti G. O. Sars, and L. minor T. and A. Scott, with a description of the male L. scotti. J Mar Biol Ass UK 20: 29-35

Nichols PD, Skerratt JH, Davidson A, Burton H, McMeekin TA (1991) The lipid composition of cultured Phaeocystis pouchetii: Signatures for food-web, biogeochemical and environmental studies in Antarctica and the Southern Ocean. Phytochemistry 30: 3209-3214

Nicol S, Stolp M (1989) Sinking rates of cast exoskeletons of Antarctic krill (Euphausia superba Dana) and their role in the vertical flux of particulate matter and fluoride in the Southern Ocean. Deep-Sea Res 36: 1753-1762

Nishida S (1986) Nanoplankton flora in the Southern Ocean, with special reference to siliceous varieties. Mem Nat Inst Polar Res, Special Issue 40: 56-68

Nøst-Hegseth E (1982) Chemical and species composition of the phytoplankton during the first spring bloom in Trondheimsfjorden, 1975. Sarsia 67: 131-141

Nöthig E-M, von Bodungen B (1989) Occurrence and vertical flux of faecal pellets of probably protozoan origin in the southeastern Weddell Sea (Antarctica). Mar Ecol Prog Ser 56: 281-289

O'Kelly JC (1974) Inorganic nutrients. In: Stewart WDP (ed) Algal Physiology and Biochemistry. Blackwell Scientific Publications, Oxford, p 610-635

Orton JH (1923) The so-called "baccy-juice" in the waters of the Thames oyster-beds. Nature 111: 773

Owens NJP, Cook D, Colebrook M, Hunt H, Reid, PC (1989) Long term trends in the occurrence of Phaeocystis sp. in the north-east Atlantic. J Mar Biol Ass UK 69: 813-821

Paerl HW (1988) Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnol Oceanogr 33: 823-847

Pain S (1989) Pollutants collect on continental side of North Sea, study finds. New Scientist 11: 24

Palmisano AC, SooHoo JB, SooHoo SL, Kottmeir ST, Craft LL, Sullivan CW (1986) Photoadaptation in Phaeocystis pouchetii advected beneath annual sea ice in McMurdo Sound, Antarctica. J Plank Res 8: 891-906

Palmisano AC, Sullivan CW (1983) Sea ice microbial communities (SIMCO) I. Distribution, abundance and primary production of ice microalgae in McMurdo Sound, Antarctica in 1980. Polar Biol 2: 171-177

Palmisano AC, Sullivan CW (1985) Pathways of photosynthetic carbon assimilation in sea-ice microalgae from McMurdo Sound, Antarctica. Limnol Oceanogr 30: 674-678

Paperzak L (1993) Daily irradiance governs growth rate and colony formation of Phaeocystis (Prymnesiophyceae). J Plankton Res 15: 809-821

Parke M, Dixon PS (1968) Check-list of British marine algae - 2nd revision. J Mar Biol Ass UK 48: 783-832

Parke M, Green JC, Manton I (1971) Observations on the fine structure of the zoids of the genus Phaeocystis (Haptophyceae). J Mar Biol Ass UK 51: 927-941

Passow U, Wassmann P (1994) On the trophic fate of Phaeocystis pouchetii (Hariot): IV. The formation of marine snow by P. pouchetii. Mar Ecol Prog Ser 104: 153-161

Pearce F (1988) Plankton shares the blame for sulphur pollution. New Scientist 117: 25

Perrin R, Lu P, Marchant HJ (1987) Seasonal variation in marine phytoplankton and ice algae at a shallow Antarctic coastal site. Hydrobiologia 146: 33-46

Pienaar RN, Cooper GA (1991) Ultrastructure of the motile disc-bearing phase of Phaeocystis. J Phycol 27: 59

Pieters H, Kluytmans JH, Zandee DI, Cadée GC (1980) Tissue composition and reproduction of Mytilus edulis in relation to food availability. Neth J Sea Res 14: 349-361

Pomeroy LR, Deibel D (1986) Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science 233: 359-361

Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25: 943-948

Porter KG, Sherr EB, Sherr BF, Pace M, Sanders RW (1985) Protozoa in planktonic food webs. J Protozool 32: 409-415

Pouchet MG (1892) Sur une algue pélagique nouvelle. Compte Rendus Hebdomadaires Seances et Memoires Societe de Biol 44: 34-36

Prézelin BB, Boucher PB, Smith RC (1993) Daytime kinetics of UV-A and UV-B inhibition of photosynthetic activity in Antarctic surface waters. In: Yamamoto H,

Smith CM (eds) Photosynthetic responses to the environment. Proceedings of the American society of plant physiology, Rockville, Md, 8 p 150-155

Prézelin BB, Smith RC (1993) Polythene bags and solar radiation: response. Science 259: 534-535

Prézelin BB, Tilzer MM, Schofield O, Haese C (1991) The control of the production process of phytoplankton by the physical structure of the aquatic environment with special reference to its optical properties. Aquat Sci 53: 136-186

Priddle J (1990) The Antarctic planktonic ecosystem. In: Medlin LK, Priddle J (eds) Polar marine diatoms. British Antarctic Survey, Cambridge, p 25-34

Priddle J, Heywood RB, Theriot E (1986) Some environmental factors influencing phytoplankton in the Southern Ocean around South Georgia. Polar Biol 5: 65-79

Priscu JC, Priscu LR, Palmisano AC, Sullivan CW (1990) Estimation of neutral lipid levels in Antarctic sea ice microalgae by nile red fluoresence. Antarct Sci 2: 149-155

Puskeppeleit M, Quintern LE, El Naggar S, Schott J-U, Eschweiler U, Horneck G, Bücker H (1992) Long–term of solar UV radiation in Antarctica with spores of Bacillus subtilis. App Env Microbiol 58: 2355-2359

Putt M, Miceli G, Stoecker DK (1994) Association of bacteria with Phaeocystis sp. in McMurdo sound, Antarctica. Mar Ecol Prog Ser 105: 179-189

Reid PC (1975) Large scale changes in North Sea phytoplankton. Nature 257: 217-219

Renger G, Volker M, Echert HJ, Fromme R, Holm-Veit S, Grader P (1989) On the mechanism of photosystem II deterioration by UV-B radiation. Photochem Photobiol 49: 97-105

Reynolds CS, Thompson JM, Ferguson AJD, Wiseman SW (1982) Loss processes in the population dynamics of phytoplankton maintained in closed sysems. J Plankton Res 4: 561-600

Richards FA, Thompson TG (1952) The estimation and characterization of plankton populations by pigment analysis 2. A spectrophotometric method for plankton pigment. J Mar Res 11: 156-172

Richardson MG, Whitaker TM (1979) An antarctic fast-ice food chain: Observations on the interaction of the amphipod Pontogeneia antarctica Chevreux with ice-associated micro-algae. Br Antarct Surv Bull 47: 107-115

Rick H-J, Aletsee L (1989) The distribution of the haptophytes Phaeocystis pouchetii (Hariot) Lagerheim and Phaeocystis globosa Scherffel in the North Sea during May, June 1986 and February, March 1987. Meeresforsch 32: 169-176

Riebesell U (1993) Aggregation of Phaeocystis during phytoplankton spring blooms in the southern North Sea. Mar Ecol Prog Ser 96: 281-289

Riebesell U, Schloss I, Smetacek V (1991) Aggregation of algae released from sea ice: implications for seeding and sedimentation. Polar Biol 11: 239-248.

Riegman R, Colijn F, Malschaert JFP, Kloosterhuis HT, Cadée, G.C. (1990) Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics. Neth J Sea Res 26: 53-60

Riegman R, Noordeloos AM, Cadée, GC (1992) Phaeocystis blooms and eutrophication of the continental coastal zones of the North Sea. Marine Biol 112: 479-484

Rivkin, RB, Putt M, Alexander SP, Meritt D, Gaudet L (1989) Biomass and production in polar planktonic and sea ice microbial communities: a comparative study. Mar Biol 101: 273-283

Roberts L (1989) Does the ozone hole threaten Antarctic life? Science 244: 288-289

Rogers SI, Lockwood SJ (1990) Observations on coastal fish fauna during a spring bloom of Phaeocystis pouchetii in the eastern Irish Sea. J Mar Biol Ass UK 70: 249-253

Ross RM, Quetin LB (1986) How productive are Antarctic krill. BioScience 36: 264-269

Rousseau V, Mathot S, Lancelot C (1990) Calculating carbon biomass of Phaeocystis sp. from microscopic observations. Mar Biol 107: 305-314

Rousseau V, Vaulot D, Casotti R, Cariou V, Lenz J, Gunkel J, Baumann, M (1994) The life cycle of Phaeocystis (Prymnesiophyceae): evidence and hypotheses. J Mar Syst 5: 23-40

Roy CR, Gies HP, Tomlinson DW, Lugg DL (1994) Effects of ozone depletion on the ultraviolet radiation environment at the Australian stations in Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 1-16

Ryan KG (1992) UV radiation and photosynthetic production in Antarctic sea ice microalgae. J Photochem Photobiol B: Biol 13: 235-240

Ryan KG, Beaglehole D (1994) Ultraviolet radiation and bottom-ice algae: Laboratory and field studies from McMurdo Sound, Antarctica. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 229-242

Saijo Y, Kawashima T (1964) Primary production in the Antarctic Ocean. J Oceanogr Soc Jpn 19:190-196

Sakshaug E, Skjoldal HR (1989) Life at the ice edge. Ambio 18: 60-67

Sargent JR, Eilertsen HC, Falk-Petersen S, Taasen, JP (1985) Carbon assimilation and lipid production in phytoplankton in the northern Norwegian fjords. Mar Biol 85: 109-116

Sargent JR, Falk-Petersen S (1989) The lipid biochemistry of Calanus. Hydrobiologia 167/168: 101-114

Sargent JR, Whittle KJ (1981) Lipids and hydrocarbons in the food web. In: Longhurst A (ed) Analysis of marine ecosystems. Academic Press, London, p 491-533

Satoh H, Fikami K, Watanabe K, Takahashi E (1989) Seasonal changes in heterotrophic bacteria under fast ice near Syowa station, Antarctica. Can J Microbiol 35: 329-333

Savage RE (1930) The influence of Phaeocystis on the migrations of the herring. Fish Invest Ser 2 12: 1-14

Savage RE (1932) Phaeocystis and herring shoals. J Ecol 20: 326-340

Scherer S, Chen TW, Böger P (1988) A new UV-A/B protecting pigment in the terrestrial cyanobacterium Nostoc commune. Plant Physiol 88: 1055-57

Scherffel A (1899) Phaeocystis globosa n. sp. (Vorläufige Mitteilung). Ber dt Bot Ges 17: 317-318

Scherffel A (1900) Phaeocystis globosa nov. spec. nebst einegen betrachtungen über die phylogenie niederer, insbesonderer brauner organismen. Wiss Meeresunters NF Abt Helgoländ 4: 1-28

Schindler DW, Schmidt RV, Ried RA (1972) Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. J Fish Res Bd Canada 29: 1627-1631

Schloss I, Estrada M (1994) Phytoplankton composition in the Weddell–Scotia confluence area during spring in relation to hydrography. Polar Biol: 77-90

Schnack SB (1983) On the feeding of copepods on Thalassiosira partheneia from the northwest African upwelling area. Mar Ecol Prog Ser 11: 49-53

Schnack SB, Smetacek V, Bodungen Bv, Stegmann P (1984) Utilization of phytoplankton by copepods in Antarctic waters during spring. In: Gray J (ed) Proceedings of the 18th European marine biology symposium, Oslo. John Wiley, London, p 275-287

Schofield O, Kroon BMA, Prézelin BB (1995) Impact of ultraviolet-B radiation on photosystem II activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities. J Phycol 31: 703-715

SCOPE (1993) Environmental effects of ultraviolet radiation on global ecosystems. Scientific Committee on Problems of the Environment. Secretariate, Paris, p 1-47

Scott F, Marchant HJ (in prep.) Seasonal variation in the particle size spectrum in relation to planktonic components at an Antarctic coastal site. Polar Biol

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

Sebastian C, Scheuerlein R, Häder D-P (1994) Graviperception and motility of three Prorocentrum strains impaired by solar and artificial ultraviolet radiation. Mar Biol 120: 1-7

Seckmeyer G, McKenzie RL (1992) Increased ultraviolet radiation in New Zealand (45°S) relative to Germany (48°N). Nature 359: 135-137

Setlow R (1974) The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. Proc Natl Acad Sci USA 71: 3363-3366

Sherr BF, Sherr EB, Pedros-Alio C (1989) Simultaneous measurement of bacterioplankton production and protozoan bactivory in estuarine water. Mar Ecol Prog Ser 54: 209-219

Sherr GB (1988) Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature 335: 348-351

Shibata K (1969) Pigments and UV–absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. Pl Cell Physiol 88: 1055-1057

Shibata H, Baba K, Ochiai H (1991) New UV irradiation induced shock proteins in Anacystis nidulans R-2; possible role of oxygen. Plant Cell Physiol 32:771-776

Shick JM, Lesser MP, Dunlap WC, Stochaj WR, Chalker BE, Won JW (1995) Depth-dependent responses to solar ultraviolet radiation and oxidative stress in the zooxanthellate coral Acropora microphthalma. Mar Biol: 122 41-51

Sieburth JMcN (1960) Acrylic acid, an "antibiotic" principle in Phaeocystis blooms in Antarctic waters. Science 132: 676-677

Sieburth JMcN (1961) Antibiotic properties of acrylic acid. A factor in gastrointestinal antiobiosis of polar marine animals. J Bacteriol 82: 72-79

Sieburth JMcN (1964) Antibacterial substances produced by marine algae. In: Developments in industrial microbiology, Society for Industrial Microbiology, Washington, DC, p 124-134

Sieburth, JMcN (1979) Sea Microbes. Oxford University Press, London

Silver MW, Mitchell JG, Ringo DL (1980) Siliceous nanoplankton II. Newly discovered cysts and abundant choanoflagellates from the Weddell Sea, Antarctica. Mar Biol 58: 211-217

Sivalingam PM, Ikawa T, Yokohama Y, Nisizawa K (1974) Distribution of 334 UV-absorbing-substances in the algae, with special regard of its possible physiological roles. Bot Mar 17: 23-29

Sivalingam PM, Ikawa T, Nisizawa K (1976) Physiological roles of a substance 334 in algae. Bot Mar 19: 9-21

Sivalingam PM, Nisizawa K (1990) Ozone hole and its correlation with the characteristic UV–absorbing substance in marine algae. Jpn J Phycol 38: 365-370

Skreslet S (1988) Bouyancy of Phaeocystis pouchetii (Hariot) Lagerheim. J Exp Mar Biol Ecol 199: 157-166

Slezak DM, Puskaric S, Herndl G (1994) Potential role of acrylic acid in bacterioplankton communities in the sea. Mar Ecol Prog Ser 105: 191-197

Smayda TJ (1973) The growth of Skeletomena costatum during a winter–spring bloom in Narragansett Bay, R.I. Norw J Bot 20: 219-247

Smayda TJ (1980) Phytoplankton species sucession. In: Morris IO (ed) The physiological ecology of phytoplankton. Blackwell Scientific Publications, London, p 493-570

Smith RC (1989) Ozone, middle ultraviolet radiation and the aquatic environment. Photochem Photobiol 50: 459-469

Smith RC, Baker KS (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem Photobiol 29: 311-323

Smith RC, Baker KS (1989) Stratospheric ozone, middle ultraviolet radiation and phytoplankton productivity. Oceanography 2: 4-10

Smith RC, Baker KS, Holm-Hansen O, Olson R (1980) Photoinhibition of photosynthesis in natural waters. Photochem Photobiol 31: 585-592

Smith RC, Prezelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992)

Ozone Depletion : Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255: 952-959

Smith WO Jr (1987) Phytoplankton dynamics in marginal ice zones. Oceanogr Mar Biol Ann Rev 25: 11-38

Smith WO Jr (1987) Phytoplankton dynamics in marginal ice zones. Oceanogr Mar Biol Annu Rev 25: 952-959

Smith WO Jr (1993) Nitrogen uptake and new production in the Greenland Sea: The spring Phaeocystis bloom. J Geophysical Res 98:4681-4688

Smith WO Jr, Codispoti LA, Nelson DM, Manley T, Buskey EJ, Niebauer HJ, Cota GF (1991) Importance of Phaeocystis blooms in the high-latitude ocean carbon cycle. Nature 352: 514-516

Smith WO Jr, Keene NK, Comiso JC (1988) Interannual variability in estimated primary productivity of the antarctic marginal ice edge zone. In: Sahrhage D (ed) Antarctic Ocean and Resources Variability. Springer-Verlag, Berlin, p 131-139

Smith WO Jr, Nelson DM (1985) Phytoplankton bloom produced by a receding ice edge in the Ross Sea: spatial coherence with the density field. Science 227: 163-166

Smith WO Jr, Nelson DM (1986) Importance of ice edge phytoplankton production in the Southern Ocean. BioScience 36: 251-257

Solomon S (1990) Progress towards a quantitative understanding of Antarctic ozone depletion. Nature 347: 347-354

SooHoo JB, Palmisano AC, Kottmeier ST, Lizotte MP, SooHoo SL, Sullivan CW (1987) Spectral light absorbance and quantum yield of photosynthesis in sea ice microalgae and a bloom of Phaeocystis pouchetii from McMurdo Sound, Antarctica. Mar Ecol Prog Ser 39: 175-189

Sournia A (1988) Phaeocystis (Prymnesiophyceae): How many species? Nova Hedwigia 47: 211-217

Squire VA (1990) Sea ice: its formation, distribution and properties. In: Medlin LK, Priddle J (eds) Polar marine diatoms. British Antarctic Survey, Cambridge, p 3-8

Statham JA, McMeekin TA (1994) Survival of faecal bacteria in Antarctic coastal waters. Antarct Sci 6: 333-338

Steemann Nielsen E (1952) The use of radio-active carbon for measuring organic production in the sea. J Cons Perm Int Explor Mer 18: 117-140

Stefánsson U, Ólafsson J (1990) Anomolous silicate-nitrate relationships associated with Phaeocystis pouchetii blooms. Eos 71: 66

Stefels J, Dijkhuizen L, Gieskes WWC (1995) DMSP–lyase in a spring phytoplankton bloom of the Dutch coast, related to Phaeocystis sp. abundance. Mar Ecol Prog Ser 123: 235-243

Stefels J, van Boekel WHM (1993) Production of DMS from dissolved DMSP in axenic cultures of the marine phytoplankton species Phaeocystis sp. Mar Ecol Prog Ser 97: 11-18

Stoecker DK, Buck KR, Putt M (1992) Changes in the sea-ice brine community during the spring–summer transition, McMurdo Sound, Antarctica. I. Photosynthetic protists. Mar Ecol Prog Ser 84: 265-278

Stoecker DK, Michaels AE, Davis LH (1987) Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature 326: 790-792

Stolarski R, Bojkov R, Bishop L, Zerefos C, Staehelin J, Zawodny J (1992) Measured trends in stratospheric ozone. Science 256: 342-349

Stolarski RS, Krueger AJ, Schoeberl MR, McPeters RD, Newman PA, Alpert JC (1986) Nimbus 7 satellite measurements of the springtime Antarctic ozone decrease. Nature 322: 808-811

Stretch JJ, Hammer PP, Michel WC, Cook J, Sullivan CW (1988) Foraging behaviour of krill Euphausia superba on sea ice microalgae. Mar Ecol Prog Ser 44: 131-139

Strid A, Chow WS, Anderson JM (1990) Effect of supplementary ultraviolet-B on photosynthesis in Pisum sativum. Biochem Biophys Acta 1020: 260-268

Sullivan CW, McClain CR, Comiso JC, Smith WO Jr (1988) Phytoplankton standing crops within an Antarctic ice edge assessed by satellite remote sensing. J Geophys Res 93: 12487-12498

Sullivan CW, Palmisano, SooHoo JB (1984) Influence of sea ice and sea ice biota on the downwelling irradiance and spectral composition of light in McMurdo Sound. Ocean Optics 7: 159-165

Takahashi E, Watanabe K, Satoh H (1986) Siliceous cysts from Kita-No-Seto Strait, North of Syowa Station, Antarctica. Mem Nat Inst Polar Res Special Issue 40: 84-95

Tande KS, Båmstedt U (1987) On the trophic fate of Phaeocystis pouchetii. I. Copepod feeding rates on solitary cells and colonies of P. pouchetii. Sarsia 72: 313-320

Tanimura Y, Fukuchi M, Watanabe K, Moriwaki K (1990) Diatoms in the water column and sea-ice in Lutzow-Holm Bay, and their preservation in the underlying sediments. Bull Natural Sci Mus. Tokyo Ser C 16: 15-39

Tefler A, Rivas JDL, Barber J (1991) β–carotene within the isolated photosystem II reaction centre: photooxidation and irreversible bleaching of this chromophore by oxidising P680. Biochem Biophys Acta 1060: 106-114

Tevini M (1993) Molecular biological effects of ultraviolet radiation. In: Tevini M (ed) UV-B radiation and ozone depletion; effects on humans, animals, plants microorganisms and materials. Lewis Publishers, Boca Raton p 1-15

Tevini M, Teramura AH (1989) UV-B effects on terrestrial plants. Photochem Photobiol 50: 479-487

Theriot E, Fryxell GA (1985) Multivariate statistical analysis of net diatom species distributions in the south western Atlantic and Indian Ocean. Polar Biol 5: 22-30

Thingstad F, Billen G (1994) Microbial degredation of Phaeocystis material in the water column. J Mar Syst 5: 55-66

Thingstad F, Martinussen I (1991) Are bacteria alive in the cold pelagic ecosystem of the Barents Sea? Polar Res 10: 255-266

Thompson PA, Harrison PJ, Parslow JS (1991) Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J Phycol 27: 351-360

Thomson BE, Worrest RC, Van Dyke H (1980) The growth response of an estuarine diatom (Melosira nummuloides [Dillw.] Ag.) to UV-B (290-320 nm) radiation. Estuaries 3: 69-72

Thomson PA, Harrison PJ, Parslow JS (1991) Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J Phycol 27: 351-360

Trodahl HJ, Buckley RG (1989) Ultraviolet levels under sea ice during the Antarctic spring. Science 245: 194-195

Truesdale RS, Kellogg TB (1979) Ross Sea diatoms: modern assemblage distributions and their relationship to ecologic, oceanographic and sedimentary conditions. Mar Micropaleontol 4: 13-31

Turner SM, Nightingale PD, Broadgate W, Liss PS (1995) The distribution of dimethyl sulfide and dimethylsulphoniopropionate in Antarctic waters and sea ice. Deep-Sea Res 42:1059-1080

Tsujiuno I, Yabe K, Sekekawa I (1980) Isolation and structure of a new amino acid, shinorine, from the red alga Chondrus yendoi Yamada et Mikami. Bot Mar 23: 65-68

UNEP (1989) Environmental effect panel report. United Nations Environment Program, Nairobi, Kenya

UNEP (1991) Environmental effects of ozone depletion: 1991 update. United Nations Environment Program, Nairobi, Kenya

USEPA (1987) An assessment of the effects of ultraviolet-B radiation on aquatic organisms. In assessing the risks of trace gases that can modify the stratosphere. United States Environment Protection Agency, EPA 400/1.87.001C, USA, 12: 1-33

Vairavamurthy A, Andreae MO, Iverson RL (1985) Biosynthesis of dimethylsulfide and dimethylpropiothetin by Hymenomonas carterae in relation to the sulfur source. Limnol Oceanogr 30: 59-70

van Bennekom AJ, Gieskes WWC, Tijssen SB (1975) Eutrophication of Dutch coastal waters. Proc R Soc Lond (B) 189: 359-374

van Boekel WHM (1991) Ability of Phaeocystis to grow on organic phosphates; direct measurement and prediction with the use of an inhibition constant. J Plankton Res 13: 959-970

van Boekel WHM (1992) Phaeocystis colony mucus components and the importance of calcium ions for stability. Mar Ecol Prog Ser 87: 301-305

van Boekel WHM, Hansen FC, Riegman R, Bak RPM (1992) Lysis induced of a Phaeocystis spring bloom and coupling with the microbial foodweb. Mar Ecol Prog Ser 81: 269-276

van Boekel WHM, Veldhuis MJW (1990) Regulation of alkaline phosphatase synthesis in Phaeocystis sp. Mar Ecol Prog Ser 61: 281-289

van Rijswijk P, Bakker C, Vink M (1989) Daily fecundity of Temora longicornis (Copepoda Calanoida) in the Oosterschelde estuary (SW Netherlands). Neth J Sea Res 23: 293-303

Veldhuis MJW (1987) The eco–physiology of the colonial alga Phaeocystis pouchetii. Rijksunive Groningen Doct

Veldhuis MJW, Admiraal W (1985) Transfer of photosynthetic products in gelatinous colonies of Phaeocystis pouchetii (Haptophyceae) and its effect on the measurement of excretion rate. Mar Ecol Prog Ser 26: 301-304

Veldhuis MJW, Admiraal W (1987) Influence of phosphate depletion on the growth and colony formation of Phaeocystis pouchetii. Mar Biol 95: 47-54

Veldhuis MJW, Admiraal W, Colijn F (1986a) Chemical and physiological changes of the phytoplankton during the spring bloom, dominated by Phaeocystis pouchetii

(Haptophyceae): Observation in Dutch coastal waters of the North Sea. Neth J Sea Res 20: 49-60

Veldhuis MJW, Colijn F, Admiraal W (1991) Phosphate utilization in Phaeocystis pouchetii (Haptophyceae). Mar Ecol 12: 53-62

Veldhuis MJW, Colijn F, Venekamp LAH (1986b) The spring bloom of Phaeocystis pouchetii (Haptophyceae) in Dutch coastal waters. Neth J Sea Res 20: 37-48

Veldhuis MJW, Colijn F, Venekamp LAH, Villerius L (1988) Phytoplankton primary production and biomass in the western Wadden Sea (the Netherlands); a comparison with an ecosystem model. Neth J Sea Res 22: 37-49

Veldhuis MJW, Venekamp LAH, Ietswaart T (1987) Availability of phosphorus sources for blooms of Phaeocystis pouchetii (Haptophyceae) in the North Sea: impact of the River Rhine. Neth J Sea Res 21: 219-229

Verity PG, Smayda TJ, Sakshaug E (1991) Photosynthesis, excretion, and growth rates of Phaeocystis colonies and solitary cells. Polar Res 10: 117-128

Verity PG, Smayda TJ (1989) Nutritional value of Phaeocystis pouchetii (Prymnesiophyceae) and other phytoplankton for Acartia spp. (Copepoda): ingestion, egg production and growth of nauplii. Mar Biol 100: 161-171

Verity PG, Villareal TA, Smayda TJ (1988a) Ecological investigations of blooms of colonial Phaeocystis pouchetii. I. Abundance, biochemical composition, and metabolic rates. J Plankton Res 10: 219-248

Verity PG, Villareal TA, Smayda TJ (1988b) Ecological investigations of blooms of colonial Phaeocystis pouchetii. II. The role of life-cycle phenomena in bloom termination. J Plankton Res 10: 749-766

Vernet M, Brody EA, Holm-Hansen O, Mitchell, BG (1994) The response of Antarctic phytoplankton to ultraviolet radiation: Absorption, photosynthesis and taxonomic composition. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p 143-158

Vernet M, Mitchell BG, Holm-Hansen O (1989) Ultraviolet radiation in Antarctic waters: response of phytoplankton pigments. Antarct J US 24: 181 - 183

Vesk M, Jeffrey SW (1987) Ultrastructure and pigments of two strains of the picoplanktonic alga Pelagococcus subviridis (Crysophyceae). J Phycol 23: 322-336

Veth C (1991) The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia–Weddell Sea. J Mar Syst 2: 451-464

Villafañe VE, Helbling EW, Holm-Hansen O, Chalker BE (1995) Acclimatization of Antarctic natural phytoplankton assemblages when exposed to solar ultraviolet radiation. J Plankton Res 17: 2295-2306

Vincent WF (1988) Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge

Vincent WF, Roy S (1993) Solar ultraviolet-B radiation and aquatic primary production: damage, protection and recovery. Environ Rev 1: 1-12

Virtue P, Nicol S, Nichols PD (1993a) Changes in the digestive gland of Euphausia superba during short–term starvation: lipid class, fatty acid and sterol content and composition. Mar Biol 117: 441-448

Virtue P, Nichols PD, Nicol S, McMinn A, Sikes EL (1993b) The lipid composition of Euphausia superba Dana in relation to the nutritional value of Phaeocystis pouchetii (Hariot) Lagerheim. Antarct Sci 5: 169-177

von Bodungen B, Smetacek VS, Tilzer MM, Zeitzschel B (1986) Primary production and sedimentation during spring in the Antarctic Peninsula region. Deep-Sea Res 33: 177-194

von Bröckel K (1981) The importance of nanoplankton within the pelagic Antarctic ecosystem. Kieler Meeresforsch 5: 61-67

von der Gathen P, Rex M, Harris NRP, Lucic D, Knudsen BM, Braathen GO, Backer HD, Fabian R, Fast H, Gil M, Kyro E, Mikkelsen IS, Rummukainen M, Stähelin J, Varotsos C (1995) Observational evidence for chemical ozone depletion over the Arctic in winter 1991-92. Nature 375: 131-134

Vosjan JH, Döhler G, Nieuwland G (1990) Effect of UV-B irradiance on the ATP content of microorganisms of the Weddell Sea (Antarctica). Neth J Sea Res 25: 391-393

Voytek MA (1989) Ominous future under the ozone hole: assessing biological impacts in Antarctica. Environmental Defence Fund, Washington, p 1-69

Voytek MA (1990) Addressing the biological effects of decreased ozone on the Antarctic environment. Ambio 19: 52-61

Walne PR (1970) Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytlius. Fish Invest, London 26: 1-62

Wang KS, Chai T-j (1994) Reduction in omega–3 fatty acids by UV-B radiation in microalgae. J Appl Phycol 6: 415-421

Wassmann P (1994) Significance of sedimentation for the termination of Phaeocystis blooms. J Mar Syst 5: 81-100

Wassmann P, Peinert R, Smetacek V (1991) Patterns of production and sedimentation in the boreal and polar Northeast Atlantic. Polar Res 10: 209-228

Wassmann P, Vernet M, Mitchell BG, Rey F (1990) Mass sedimentation of Phaeocystis pouchetii in the Bering Sea. Mar Ecol Prog Ser 66: 183-195

Watanabe K (1982) Centric diatom communities found in Antarctic sea ice. Antarct Rec 74: 119-126

Watanabe K (1988) Sea-ice microbial strands in the Antarctic coastal fast ice near Syowa Station. Jpn J Phycol 36: 221-229

Watanabe T, Kitajima C, Fujita S (1983) Nutritional value of live organisms used in Japan for the mass propagation of fish: a review. Aquaculture 34: 115-143

Wefer G, Fischer G, Fuetterer D, Gersonde R (1988) Seasonal particle flux in the Bransfield Strait, Antarctica. Deep-Sea Res 35: 891-898

Weiler CS, Penhale PA (1994) Preface. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic research series, vol 62, American Geophysical Union, Washington, DC, p xi-xii

Weisse T (1983) Feeding of calanoid copepods in relation to Phaeocystis pouchetii blooms in the German Wadden Sea area off Sylt. Mar Biol 74: 87-94

Weisse T, Grimm N, Hickel W, Martens P (1986) Dymanics of Phaeocystis pouchetii blooms in the Wadden Sea of Sylt (German Bight, North Sea). Estuar Cstl Shelf Sci 23: 171-182

Weisse T, Scheffel-Möser U (1990) Growth and grazing loss rates in single-celled Phaeocystis sp. (Prymnesiophyceae). Mar Biol 106: 153-158

Weisse T, Tande K, Verity P, Hansen F, Geiskes W (1994) The trophic significance of Phaeocystis blooms. J Mar Syst 5: 67-80

Wells ML, Mayer LM, Donard OFX, Souza Sierra MMd, Ackelson SG (1991) The photolysis of colloidal iron in the oceans. Nature 353: 248-250

Whitaker TM (1977) Sea ice habitats of Signy Island (South Orkneys) and their primary production. In: Llando GA (ed) Adaptations within Antarctic ecosystems. Gulf Publishing Co., Houston, p 75-82

Wilson DL, Smith WO Jr, Nelson DM (1986) Phytoplankton bloom dynamics of the western Ross Sea ice edge - I. Primary productivity and species specific production. Deep-Sea Res 33: 1375-1387

Wood WF (1987) Effect of solar ultra-violet radiation in the kelp Ecklonia radiata. Mar Biol 96: 143-150

Wood WF (1989) Photoadaptive responses of the tropical red alga Eucheuma striatum Schmitz (Gigartinales) to ultra-violet radiation. Aquatic Bot 33: 41-51

Worrest RC (1983) Impact of solar ultraviolet-B radiation (290-320 nm) upon marine microalgae. Physiol Plant 58: 428-434

Worrest RC (1986) The effect of solar UV-B radiation on aquatic systems: an overview. In: Titus JG (ed) Effects of changes in stratospheric ozone and global climate. U.S.

Environment Protection Agency and United Nations Environment Programme, vol 1, p 175-199

Worrest RC, Brooker D, Van Dyke H (1980) Results of a primary production study as effected by the type of glass in the culture bottles. Limnol Oceanogr 25: 360-364

Worrest RC, Häder D-P (1989) Effects of stratospheric ozone depletion on marine organisms. Env Conserv 16: 261-263

Worrest RC, Krystna KU, Scott JD (1981a) Sensitivity of marine phytoplankton to UV-B radiation: impact on a model ecosystem. Photochem Photobiol 33: 223-227

Worrest RC, Thomsen BE, Van Dyke H (1981b) Impact of UV-B radiation upon estuarine microcosms. Photochem Photobiol 33: 861-867

Worrest RC, Van Dyke H, Thomson BE (1978) Impact of enhanced simulated solar ultraviolet radiation upon a marine community. Photochem Photobiol 27, 471-478

Wright SW, Jeffrey SW (1987) Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. Mar Ecol Prog Ser 38: 259-266

Wright SW, Shearer JD (1984) Rapid extraction and high performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. J Chromatogr 294 281-296

Wulff A (1934) Über hydrographie und oberflächenplankton nebst verbreitung von *Phaeocystis* in der Deutschen Bucht im Mai 1933. Ber Dt Wiss Kommn Meeresforsch 7: 343-350

Yentsch CS, Yentsch CM (1982) The attenuation of light by marine phytoplankton with special reference to the absorption of near-UV radiation. In: Calkins J (ed) The role of solar ultraviolet radiation in marine ecosystems. Plenum, New York, p 691-706

Yin F, Grosjean D, Seinfeld J (1986) Analysis of atmospheric photooxidation mechanisms for organic sulfur compounds. J Geophys Res 91: 14417-14438

Zar JH (1984) Biostatistical Analysis. 2nd ed. Prentice Hall, New Jersey

Zepp RG, Callaghan TV, Erickson DJ (1995) Effects of increased solar ultraviolet radiation on biogeochemical cycles. Ambio 24: 181-187

Zwally HJ, Comiso JC, Parkinson CL, Campbell WJ, Carsey FD, Gloersen P (1983) Antarctic sea ice, 1973-1976: satellite passive-microwave observations. NASA SP-459, Washington, DC

APPENDIX 1

Thesis related publications

Marine Biology (1994) 119: 507–515

A. T. Davidson · D. Bramich · H. J. Marchant · A. McMinn

Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms

Received: 10 December 1993 / Accepted: 26 January 1994

Abstract Growth rate, survival, and stimulation of the production of UV-B (280 to 320 nm) absorbing compounds were investigated in cultures of five commonly occurring Antarctic marine diatoms exposed to a range of UV-B irradiances. Experimental UV-B exposures ranged from 20 to 650% of the measured peak surface irradiance at an Antarctic coastal site (0.533 J m^{-2} s^{-1}). The five diatom species (*Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida* and *Stellarima microtrias*) appear capable of surviving two to four times this irradiance. In contrast to *Phaeocystis* cf. *pouchetii*, another major component of the Antarctic phytoplankton, the concentrations of pigments with discrete UV absorption peaks in diatoms were low and did not change significantly under increasing UV-B irradiance. Absorbance of UV-B by cells from which pigments had been extracted commonly greatly exceeded that of the pigments themselves. Most of this absorbance was due to oxidisable cell contents, with the frustule providing the remainder. Survival of diatoms did not correlate with absorption by either pigments, frustules or oxidisable cell contents, indicating that their survival under elevated UV-B irradiances results from processes other than screening mechanisms.

Communicated by G. F. Humphrey, Sydney

A. T. Davidson · H. J. Marchant (✉)
Australian Antarctic Division,
Channel Highway, Kingston,
Tasmania 7050,
Australia

D. Bramich [1] · A. McMinn
Antarctic CRC and Institute of Antarctic
and Southern Ocean Studies,
University of Tasmania, Hobart,
Tasmania 7001,
Australia

Present address:
[1] Department of Zoology, University of Tasmania, Hobart, Tasmania 7001, Australia

Introduction

The seasonal depletion of stratospheric ozone over the Antarctic and the Southern Ocean is a major ecological issue, as it has been suggested that the resultant increase in UV-B (280 to 320 nm) reaching the biosphere may adversely effect Antarctic marine ecosystems (Bidigare 1989; El-Sayed et al. 1990; Vosjan et al. 1990; Voytek 1990; Karentz 1991). Numerous studies indicate that productivity of marine phytoplankton in surface waters is reduced under ambient and elevated levels of UV (e.g. Worrest et al. 1981; Bühlmann et al. 1987; Smith et al. 1992). However, Hobson and Hartley (1983) and Gala and Giesy (1991) reported only limited inhibition of primary production in lake and fjord phytoplankton assemblages by UV radiation.

UV-B penetrates to depths in excess of 50 m in Antarctis waters (Gieskes and Kraay 1990; Karentz and Lutze 1990; Smith et al. 1992). Antarctic phytoplankton bloom in the high-light, high-nutrient regime of the marginal ice zone (MIZ) where the depth of the pycnocline can be 10 m or less for periods of up to 6 d (Veth 1991). This springtime bloom in the MIZ accounts for up to 67% of primary production in Antarctic waters (Smith and Nelson 1986). The coincidence of stratospheric ozone depletion with this near-surface seasonal algal bloom may have important consequences for phytoplankton and higher trophic levels if survival and/or primary production are affected (El-Sayed et al. 1990; Voytek 1990; Karentz 1991; Marchant et al. 1991). The impact of further increases in UV-B on phytoplankton in the MIZ will depend on the residence time of organisms in this shallow mixed zone, their present tolerance, and their ability to adapt to higher levels of UV-B (Bidigare 1989; Karentz 1991).

The effect of increasing solar UV-B flux on Antarctic phytoplankton and higher trophic levels is equivocal (El-Sayed et al. 1990; Karentz 1991). Calkins and Thordardottir (1980) found that the tolerance of six high-latitude marine diatoms to UV-B was similar and concluded that most organisms would adapt to enhanced solar UV through in-

creased protective pigmentation, repair, or avoidance mechanisms. Other authors have proposed a shift in species composition favouring those species with greater tolerance of UV-B (Häder and Worrest 1991; Karentz 1991; Marchant and Davidson 1991). Such changes would be likely to affect trophic interactions and vertical carbon flux.

Information on the effects of UV-B on growth and survival of Antarctic marine phytoplankton is integral to an understanding of the impact of elevated UV-B exposure on the Southern Ocean ecosystem. The mechanisms of UV-B protection in Antarctic phytoplankton species are largely unknown, although UV-B absorbing compounds and DNA repair-mechanisms have recently been reported for some species (Karentz 1988; Bidigare 1989; Mitchell et al. 1989; Karentz et al. 1991 a, b; Marchant et al. 1991). Species-specific investigations on the impact of UV-B are necessary to predict the effect of ozone depletion on these primary producers and the consequent impact on the ecosystems for which they constitute the trophic base (Karentz 1991). Here we report the effect of UV-B exposure on the growth rates and survival of five species of Antarctic marine diatoms and whether their production of UV-B absorbing compounds is promoted by UV-B exposure.

Materials and methods

Light measurements

All measurements of irradiance were made with an International Light IL 1700 Radiometer equipped with detectors to measure photosynthetically available radiation (PAR), UV-A (320 to 400 nm) and UV-B (Fig. 1). A National Institute of Standards and Technology intercomparison package (NIST Test #534/240436-88) was used to calibrate each light sensor.

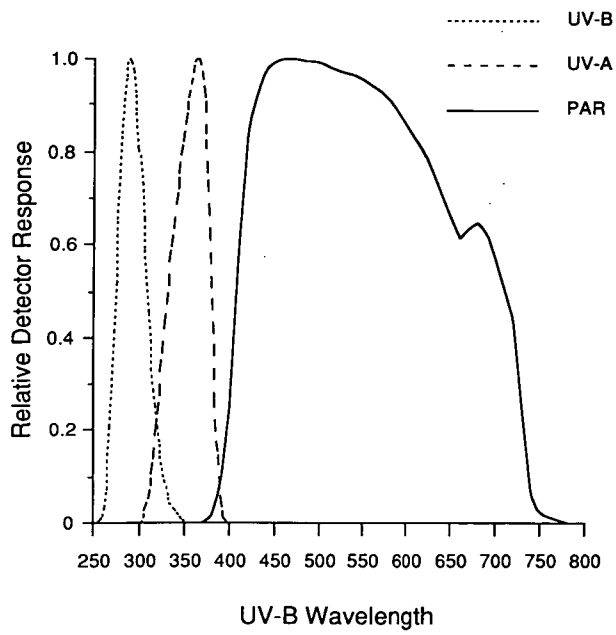


Fig. 1 Wavelength (nm) response of detectors used to measure PAR, UV-A (320 to 400 nm) and UV-B (280 to 320 nm) (redrawn from instrument specifications)

Cell isolation and culture

Unialgal cultures of the diatoms *Nitzschia lecointei* V.H., *Proboscia* (*Rhizosolenia*) *alata* (Brightwell) Sundström, *P.* (*Rhizosolenia*) *inermis* (Castracane) Jordan and Ligowski, *Thalassiosira tumida* (Jan.) Hasle, *Stellarima* (*Coscinodiscus*) *microtrias* (Ehrenberg) Hasle and Sims, *Odontella weisflogii* (Janisch) Grunow, *Nitzschia curta* (V.H.) and *Chaetoceros simplex* Ostenfeld were isolated from sea ice collected in Prydz Bay, Antarctica, during the 1990/1991 austral summer. Cultures were maintained in 250 ml glass flasks using *f*/2 growth medium (Guillard and Ryther 1962) at a temperature of 4 °C ± 2 C°. Cool-white fluorescent lights provided a PAR intensity of 11.80 m^{-2} s^{-2} (58.85 µE m^{-2} s^{-1}), with no UV-B enhancement, on a 12 h light : 12 h dark cycle.

UV-B-enhanced treatments

50 ml Lux tissue-culture flasks (which completely absorbed wavelengths below 295 nm) were filled from a single parental culture in exponential growth phase and irradiated for 24 h in a 48 h experimental period (6 h light : 12 h dark : 12 h light : 12 h dark : 6 light). Day 0 in data calculations occurs at the end of this irradiance period. Exposures were conducted in a Thermoline controlled-environment cabinet at 4 °C ± 2 C° with cool-white fluorescent tubes to provide PAR and UV-A, with UV-B provided by FS20T 12 UV-B Westinghouse sunlamps. PAR and UV-A irradiances were 12.13 ± 2.13 W m^{-2} (60.5 ± 10.4 µE m^{-2} s^{-1}) and 1.19 ± 0.68 W m^{-2}, respectively. The spectral distribution and UV-B irradiance were varied by attenuation with glass filters (Marchant et al. 1991). The zero UV-B irradiance treatment was screened by Mylar which excluded light below 320 nm. Sensors were each covered by an attenuating glass screen and a single layer of Lux culture flask to measure the experimental irradiances to which the diatoms were exposed. UV-B irradiances of 0.10 to 3.40 W m^{-2} were chosen to span the range of 20 to 650% of peak UV-B exposure as measured at an Antarctic coastal site (Casey station; 66° S) in the 1989 summer (C. Roy unpublished data). A single culture of each species was incubated at each of these irradiances and a control culture of each species was returned to culture maintenance conditions and received only PAR.

Calculation of viable cell concentration

Immediately after irradiation (Day 0), 5 or 10 ml (depending on cell concentration) of the control culture was sediment with Lugol's iodine and the concentration of cells with cytoplasmic contents (live cells) was calculated from counts over 15 replicate fields, using an Utermöhl settling tube and inverted microscope. The mean cell concentration in the control culture at Day 0 was then calculated ($N_{0\ \text{control}}$). Also on Day 0, a 5 ml aliquot of each irradiated culture and the control were inoculated into 30 ml of *f*/2 medium in a glass flask and returned to the culture maintenance conditions described above. These subcultures were incubated for up to 10 d and the concentration of live cells was counted at 2 to 4 d, intervals depending on their growth rate. The growth rate of the control culture of each species (K control) was calculated using the equation of Verity et al. (1988) (Eq. 1 below). Eq. (2) was then used to calculate the viable cell concentration on Day 0 ($N_{0\ \text{irradiated}}$) using the cell concentration for each of 15 replicate fields after ongrowth (N_t), the growth rate of the control (K control), and the time of culture ongrowth (t)

$$K = 1/t \times \log_2 \frac{N_t}{N_0}, \tag{1}$$

$$N_0\ \text{irradiated} = \frac{N_t\ \text{irradiated}}{{}_2K\ \text{control} \times t}, \tag{2}$$

$$S\% = \frac{N_0\ \text{irradiated}}{N_0\ \text{control}} \times 100, \tag{3}$$

where K = growth rate, t = number of days of growth, N_t = number of cells at time t, N_0 = number of cells immediately after irradiation (Day 0) and $S\%$ = percent survival.

The calculated viable cell concentration of each replicate field at Day 0 ($N_{0\ \text{irradiated}}$) was then converted to percent survival ($S\%$) in

comparison with the unirradiated control at Day 0 ($N_{0\ control}$) using Eq. (3). In cases where the cell number in irradiated cultures was greater than that in the control culture, computed survival could not exceed 100%. The percent survival in each replicate field was arcsine square-root transformed, the mean and standard error of the replicate fields were computed, and the mean and upper and lower confidence intervals were sine-squared to revert the data to percentages (Zar 1984).

Eq. (1) was then used to calculate the growth rate of all ongrown irradiated cultures. Growth rates were calculated for each species from the day at which the cell concentration in the culture had reached a sufficient concentration to allow statistically acceptable mean estimates (N_0) and from the concentration 4 d later (N_t). The t_0s for each species were: *Nitzschia lecointei*, Day 4; *Proboscia alata*, Day 6; *P. inermis*, Day 6; *Thalassiosira tumida*, Day 8; *Stellarima microtrias*, Day 8.

Removal of dark period from irradiance cycle

Nitzschia lecointei and *Stellarima microtrias* were exposed to the same experimental light irradiances as described above but with the dark period of the cycle removed, giving 24 h of constant illumination. These two species were chosen as they exhibited different responses to treatments which included a dark period. The same procedures were followed as described above for determining survival and growth rate.

Measurement of UV absorption

A known volume of culture was filtered through 2.5 cm diam Whatman GF/F filters. Filters were cut up into an homogeniser and 1.5 ml of 4:1, methanol:tetrahydrofuran (MeTHF) were added. The sample was then homogenised using a glass tube and teflon grinder for 30 s at ≃1000 rpm and decanted into a centrifuge tube. A further 0.5 ml of MeTHF was added to rinse the homogeniser; this was again decanted into the centrifuge tube, and the sample was centrifuged at 480×*g* for 10 min at 0 °C. The absorbance of the supernatant was measured between 250 and 800 nm using a Hewlett Packard 8450A spectrophotometer. When measurements were not carried out immediately, the extracts were stored at −120 °C for no longer than 4 wk. The wavelength of maximum UV absorbance was identified and the peak absorption height above the adjacent minima was measured for each extract. Data were then averaged over all cultures that received sublethal irradiances. Average absorbance was then normalised to chlorophyll *a* peak height at 665 nm. Cell carbon content was calculated for each species using cell concentration, volume and carbon-conversion equations of Eppley et al. (1970), and the absorbance was normalised to cell carbon concentrations (C). The amount of UV-absorbing pigment was calculated per unit C to allow comparison between species that varied in volume from ~7.90×10^2 μm³ to 1.92×10^5 μm³ for *Nitzschia lecointei* and *Proboscia inermis*, respectively. UV absorbance was also normalised to cell concentration. Regression analysis of log absorbance per cell for each species was used to ascertain whether the concentration of UV-B-absorbing compounds was promoted by increased UV-B irradiance.

Absorption by UV-B pigments, extracted cell contents and frustules was measured in exponentially growing cultures of *Nitzschia lecointei*, *Proboscia alata*, *Thalassiosira tumida*, *Odontella weisflogii*, *N. curta*, and *Chaetoceros simplex* grown in *f*/2 medium under culture maintenance conditions (as above). Seven hundred ml of each culture was centrifuged at 200×*g* for 40 min at 0 °C to concentrate the cells, and the supernatant was discarded. Two ml of 4:1, MeTHF was then added, the cells were resuspended and the intracellular pigments were allowed to extract overnight at 0 °C. The centrifugation was repeated and absorption of the supernatant was measured as above. To remove any contamination by intracellular UV-absorbing pigments, the extracted material was rinsed three times with 2.0 ml of MeTHF followed by resuspension and centrifugation at 200×*g* for 10 min at 0 °C. The material was then resuspended in a further 2.0 ml of MeTHF and the absorbance was measured as above.

To clear diatom frustules of organic contents, a known volume of the above MeTHF-extracted cell concentrate was centrifuged at 480×*g* for 10 min and the MeTHF supernatant was discarded. The sample was then digested for 24 h in 5 ml of 30% H_2O_2, and 25 g of $K_2Cr_2O_7$ were added to oxidise and clean the frustales. The solution was diluted to 15 ml with Milli Q water and centrifuged at 480×*g* for 1 h, and the supernatant again discarded. Microscopic examination of samples showed that this was sufficient to remove the cell contents from all species except *Thalassiosira tumida*; two treatments were necessary to clear the frustales of this species. Samples were resuspended twice in 15 ml of Milli Q, centrifuged at 480×*g* for 1 h, and the supernatant discarded. Finally, the cleared frustales were resuspended in a volume of MeTHF equal to that of the initial MeTHF extract, and the absorbance was measured as described above.

Results

UV-B absorbance

Only compounds with absorption >290 nm were investigated. Pigment extracts of *Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida*, *Stellarima microtrias* and *N. curta* had chlorophyll *a* absorbance peaks at 665 nm and chlorophylls and carotenoids at ≃440 nm (Fig. 2). None of the species investigated had any pronounced absorbance peaks in the UV-B region of the spectrum. There was, however, increasing background absorption in the UV region of the spectrum and distinct absorbance peaks between 325 and 342 nm for each species (Table 1), the shoulder of which absorbed at UV-B wavelength (Fig. 2). The ratio of the UV-absorbing compound peak-height to that of chlorophyll *a* at 665 nm for the diatoms was ≤2.1:1. Most of the absorption was at UV-A wavelengths, and absorption in the UV-B region at the shoulder of these peaks was much less.

As the concentration of chlorophyll *a* can change in response to UV-B exposure (Bidigare 1989), it was not used in normalising UV-B induced changes. Log absorbances per unit cell C for each species over the range of UV-B irradiances is shown in Fig. 3. Data from Antarctic *Phaeocystis* (Marchant et al. 1991) using similar methods is included for comparison. Regression analysis of the UV absorbance peak-height per cell against sublethal irradiance showed that increased UV-B flux elicited no significant response in UV absorbance in any diatom, and the *F*-test showed that the regression slopes were not significantly different from zero (Table 2).

Absorption of MeTHF-insoluble material by *Nitzschia lecointei*, *Proboscia alata*, *Odontella weisflogii*, *N. curta* and *Chaetoceros simplex* (Fig. 4 A–B, D–F) gradually decreased with increasing wavelength while that of *Thalassiosira tumida* remained approximately constant (Fig. 4 C). Absorption by cleared frustules of each species also decreased with increasing wavelength, but only accounted for between 13 and 29% of the total UV-B absorption by the cells (Figs. 4 and 5). Total cellular UV-B absorption per μg cell carbon varied between 3.4×10^{-5} for *T. tumida* to 8.2×10^{-4} for *N. lecointei* (Fig. 5). MeTHF-soluble pigments comprised between 12 and 26% of this absorption. The exception was *T. tumida*, in which it accounted for 45% of UV-B absorption. The majority of UV-B absorp-

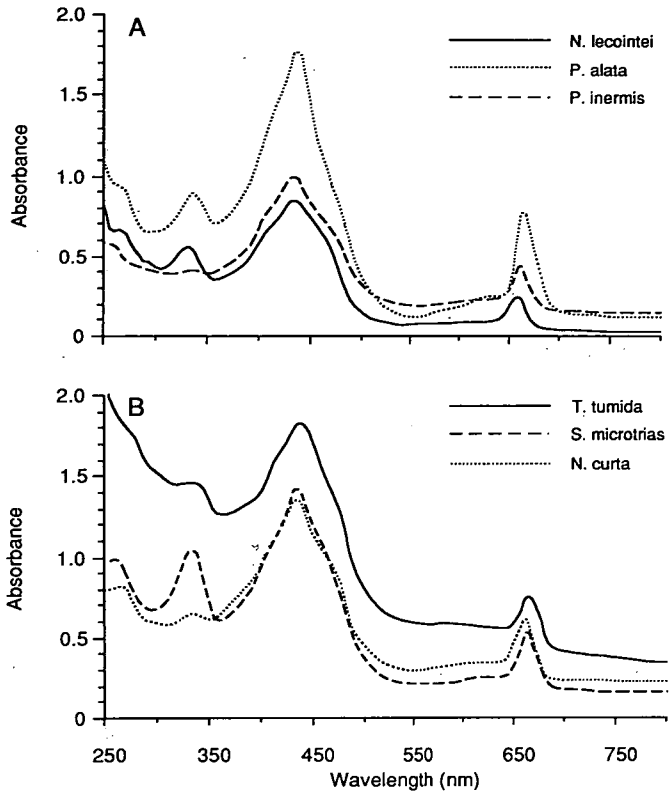


Fig. 2 **A** *Nitzschia lecointei*, *Proboscia lata* and *P. inermis*; **B** *Thalassiosira tumida*, *Stellarima microtrias* and *N. curta*. Absorbance spectra of extracts in 80% menthanol:20 tetrahydrofuran between 250 and 800 nm for control cultures

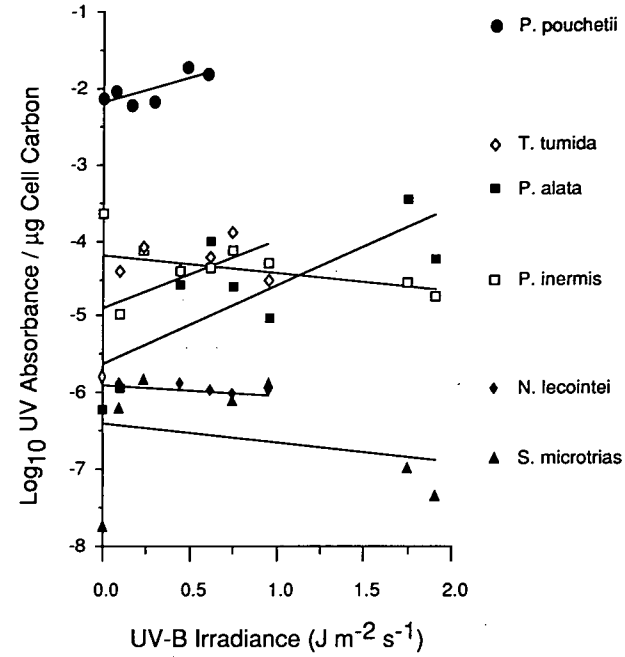


Fig. 3 *Proboscia inermis*, *Nitzschia lecointei*, *Thalassiosira tumida*, *Proboscia alata*, *Stellarima microtrias* and *Phaeocystis* cf. *pouchetii*. Log peak UV absorbance per unit cell carbon as a function of sublethal UV-B irradiances. (Data for *Phaeocystis* cf. *pouchetti* from Marchant et al. 1991)

Table 1 UV-absorbing compounds in Antarctic marine diatoms and *Phaeocystis* cf. *pouchetti* (*Phaeocystis*), showing wavelength of peak UV-absorbance, ratio of UV-absorbing compound peak-height to chlorophyll *a* peak-height at 665 nm, and UV absorbance per µg cell carbon. *Phaeocystis* calculated from data in Marchant et al. (1991). Data are mean values of all sublethal irradiances

Species	Peak absorbance (nm)	UV abs : chl *a* ratio	UV abs μg^{-1} cell C
Nitzschia lecointei	325	0.9	1.10×10^{-6}
Proboscia alata	336	1.7	6.86×10^{-5}
Proboscia inermis	340	2.1	6.17×10^{-5}
Thalassiosira tumida	342	1.2	5.08×10^{-5}
Stellarima microtrias	342	1.8	5.91×10^{-7}
Phaeocystis	323	27.5	1.04×10^{-2}

Table 2 Regression statistics obtained by linear regression of UV absorbance per cell against sublethal UV-B irradiance for Antarctic marine diatoms

Species	*P*(*r*)	*P*(*F*)
Nitzschia lecointei	0.2 >×>0.1	0.1955
Proboscia alata	0.2>×>0.1	0.1009
Proboscia inermis	0.5 >×>0.2	0.2732
Thalassiosira tumida	0.5 >×>0.2	0.2542
Stellarima microtrias	>0.50	0.5851

tion was due to MeTHF-insoluble cell contents, except in *T. tumida* where the proportion was slightly less than that of the MeTHF-soluble material (Fig. 5).

UV-B response: survival and growth rate

Survival of diatoms exposed to UV-B differed between species (Fig. 6). Diatoms screened with Mylar received no UV-B (irradiance 0; Fig. 6), but did receive unattenuated UV-A and exhibited low survival (9 to 32%). Survival of *Nitzschia lecointei*; *Proposcia alata* and *P. inermis* at sublethal irradiances approximated 100% survival (Fig. 6A–C) but that of *Thalassiosira tumida* and *Stellarima microtrias* at sublethal irradiances ranged from 51 to 85% and 59 to 75%, respectively (Fig.6D, E). At 1.75 J m^{-2} s^{-1}, the survial of *N. lecointei*, and *P. alata* fell to 17 and 14% respectively and survival was negligible at 3.4 J m^{-2} s^{-1} (Fig. 6A, B). No significant change in survival of *P. inermis*, *T. tumida* and *S. microtrias* occurred until a UV-B irradiance

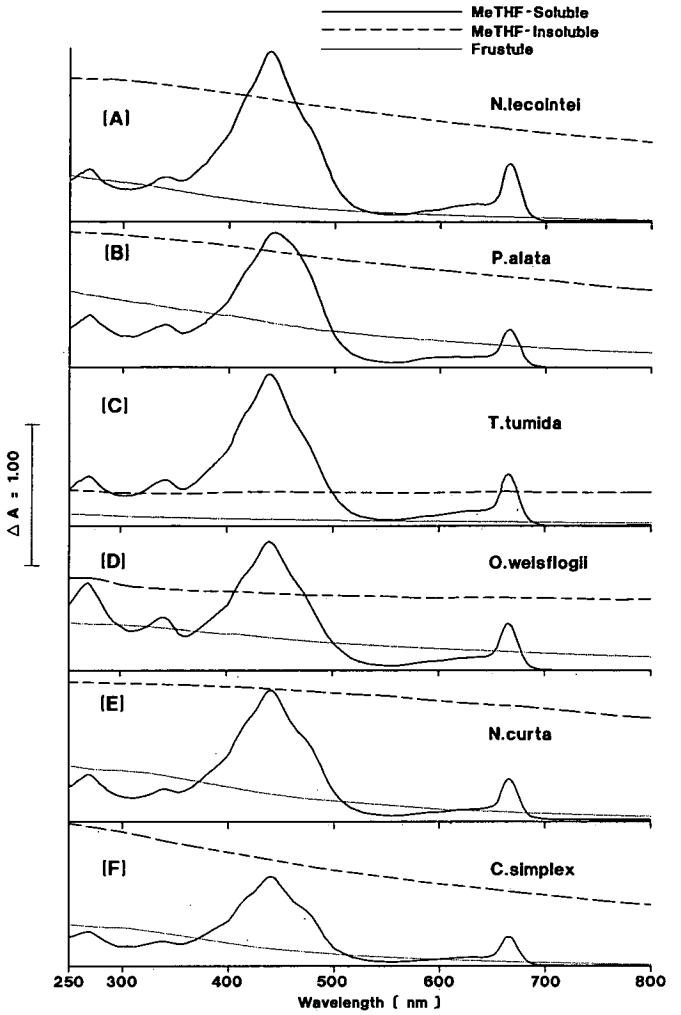


Fig. 4 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *Thalassiosira tumida*, **D** *Odontella weisflogii*, **E** *Nitzschia curta*, **F** *Chaetoceros simplex*. Absorbance spectra of extracts in 80% methanol:20 tetrahydrofuran for cultures grown under maintenance conditions. Cells were extracted with MeTHF and insoluble material was oxidised to clear frustules. Absorbance by MeTHF-soluble compounds, MeTHF-insoluble matter and cleared frustules and absorbance between 250 and 800 nm are shown

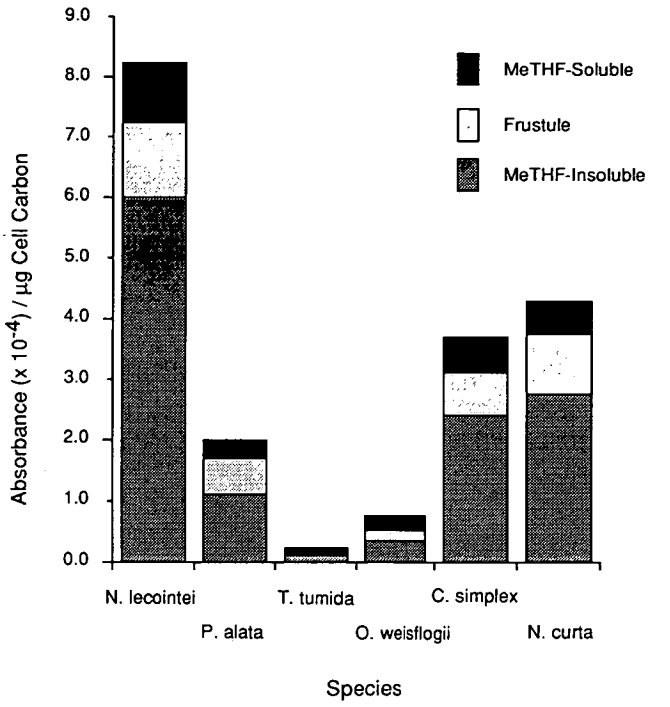


Fig. 5 *Nitzschia lecointei*, *Proboscia alata*, *Thalassiosira tumida*, *Odontella weisflogii*, *Chaetoceros simplex* and *Nitzschia curta*. Average UV-B absorbance of MeTHF-soluble compounds, MeTHF insoluble matter and cleared frustules per µg cell carbon

of 3.4 J m^{-2} s^{-1}, at which irradiance their survival fell to 25% (Fig. 6C, D, E). In contrast, the survival of Antarctic colonial *Phaeocystis* cf. *pouchetti* (Fig. 6F) (Thereafter referred to as *Phaeocystis*) was reduced to 30% at a UV-B irradiance of 1.0 J m^{-2} s^{-1}, with survival reduced to 0% at a UV-B irradiance of 2.1 J m^{-2} s^{-1} (Marchant et al. 1991).

Regression analysis showed that there was no significant relationship between growth rate after irradiation of the diatoms and the UV-B irradiance they received (Table 3). The growth rate of *Nitzschia lecointei* appeared to decline as UV-B irradiance increased, but, this was not observed until the highest irradiance (3.4 J m^{-2} s^{-1}; Fig. 7). Growth rates for irradiated cultures of *Proboscia alata*, *P. inermis*, *Thalassiosira tumida*, and *Stellarima microtrias* were comparable to those occurring in the PAR control.

Dark-period removal

Removal of the dark period from the irradiance of both *Nitzschia lecointei* and *Stellarima microtrias* elicited a different survival response (Fig. 8) from those treatments incorporating a dark period. *N. lecointei* cells survived an irradiance incorporating dark periods of 1.75 J m^{-2} s^{-1} (Fig. 6A), but survived all but the maximum irradiance when exposed without dark periods (Fig. 8A). In contrast to *N. lecointei*, survival of *S. microtrias* during irradiation including a dark period did not decline significantly until an irradiance of 3.2 J m^{-2} s^{-1}. When the dark period was removed, survival of *S. microtrias* declined to 23% at an irradiance of 1.75 J m^{-2} s^{-1}. In addition, survial of *S. microtrias* over the lower range of UV-B irradiances was ≃ 20% lower in exposures with a dark period (Fig. 6E) than in those without (Fig. 8B).

Discussion

This study was structured to approach natural conditions, so that some insights into the basic responses of diatoms

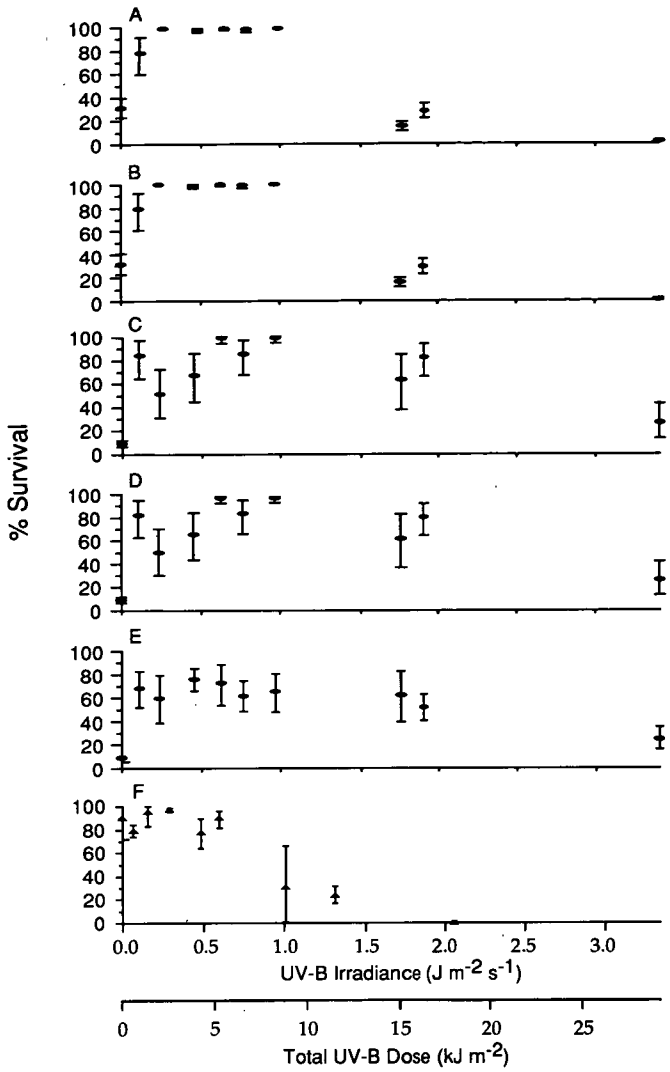


Fig. 6 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *P. inermis*, **D** *Thalassiosira tumida*, **E** *Stellarima microtrias*, **F** *Phaeocystis* cf. *pouchetti*. Percent survival of diatoms irradiated for 24 h of a 48 h period as a function of UV-B irradiance. (Data for *Phaeocystis* from Marchant et al. 1991). Errors bars represent standard errors calculated from Zar (1984)

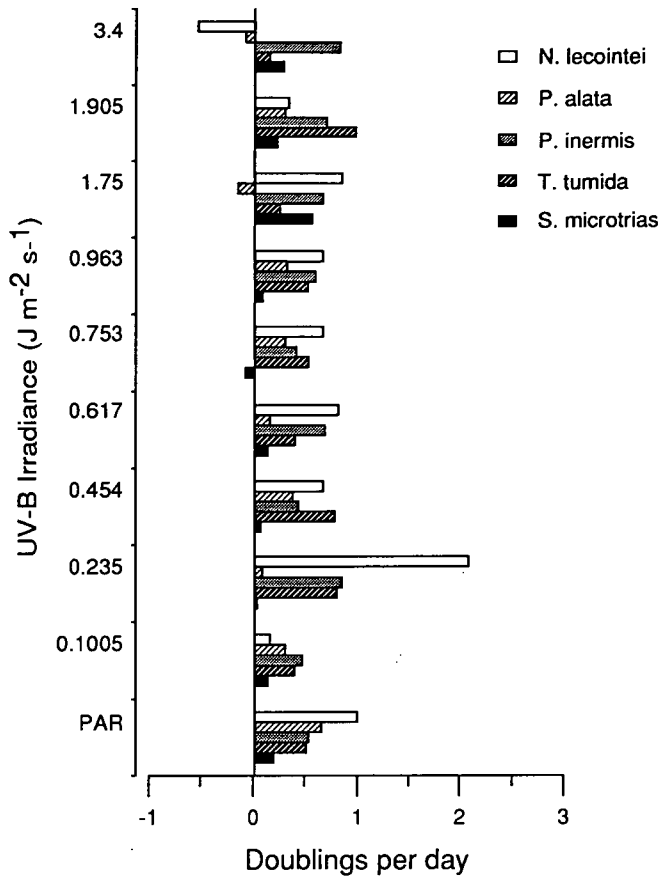


Fig. 7 *Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida* and *Stellarima microtrias*. Growth rate of exponentially growing culture after exposure to 12.13±2.13 W m^{-2} (60.5±10.6 µE m^{-2} s^{-1}) PAR, 1.19±0.68 W m^{-2} UV-A, and various UV-B irradiances

Table 3 Regression statistics obtained by linear regression of post-irradiation growth-rate against UV-B irradiance for Antarctic marine diatoms

Species	$P(r)$
Nitzschia lecointei	0.1 <×<0.05
Proboscia alata	0.2 <×<0.1
Proboscia inermis	0.1 <×<0.05
Thalassiosira tumida	0.5 <×<0.2
Stellarima microtrias	0.2 <×<0.1

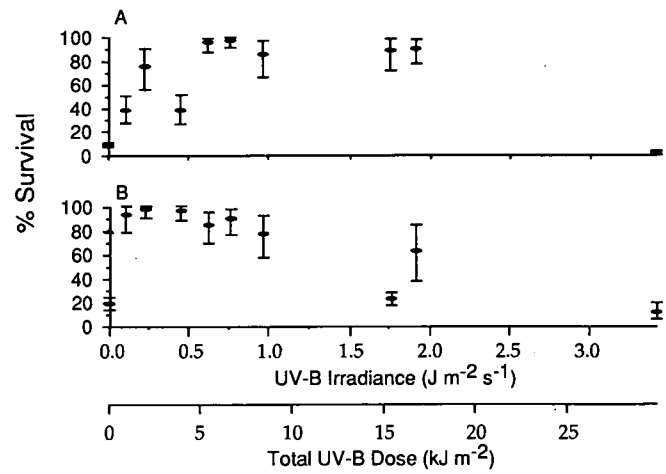


Fig. 8 **A** *Nitzschia lecointei*, **B** *Stellarima microtrias*. Percent survial after 24 h continuous UV-B irradiation. Errors bars represent standard errors calculated from Zar (1984)

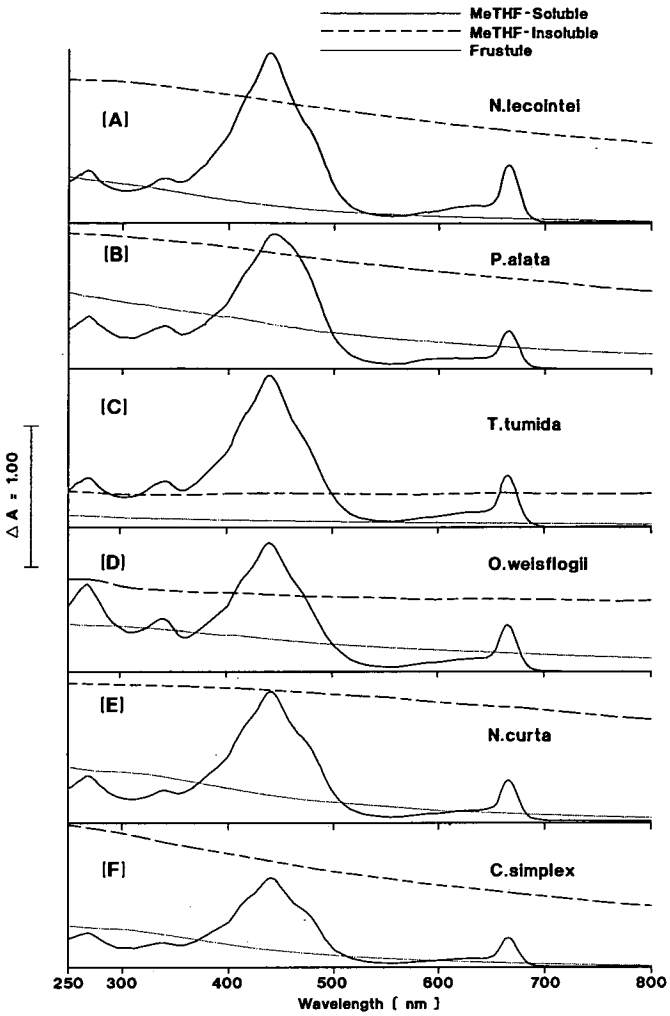


Fig. 4 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *Thalassiosira tumida*, **D** *Odontella weisflogii*, **E** *Nitzschia curta*, **F** *Chaetoceros simplex*. Absorbance spectra of extracts in 80% methanol:20 tetrahydrofuran for cultures grown under maintenance conditions. Cells were extracted with MeTHF and insoluble material was oxidised to clear frustules. Absorbance by MeTHF-soluble compounds, MeTHF-insoluble matter and cleared frustules and absorbance between 250 and 800 nm are shown

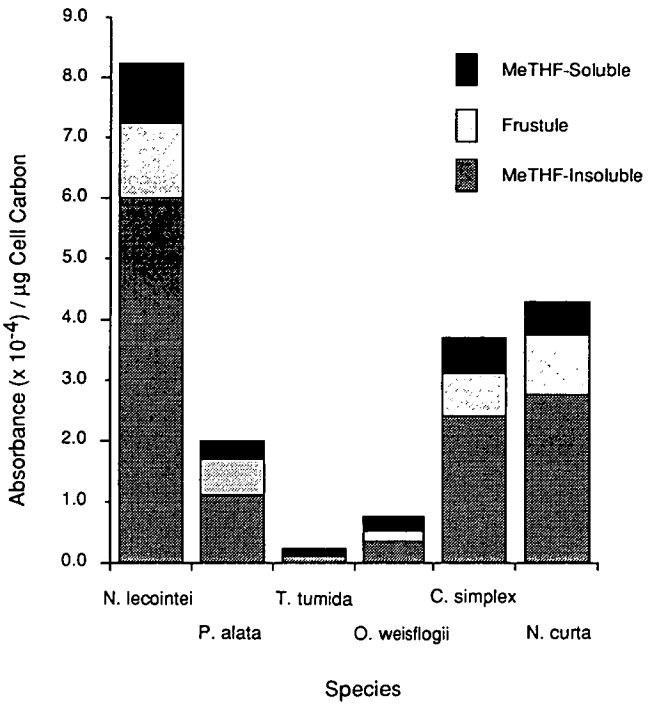


Fig. 5 *Nitzschia lecointei*, *Proboscia alata*, *Thalassiosira tumida*, *Odontella weisflogii*, *Chaetoceros simplex* and *Nitzschia curta*. Average UV-B absorbance of MeTHF-soluble compounds, MeTHF insoluble matter and cleared frustules per µg cell carbon

of 3.4 J m^{-2} s^{-1}, at which irradiance their survival fell to 25% (Fig. 6C, D, E). In contrast, the survival of Antarctic colonial *Phaeocystis* cf. *pouchetti* (Fig. 6F) (Thereafter referred to as *Phaeocystis*) was reduced to 30% at a UV-B irradiance of 1.0 J m^{-2} s^{-1}, with survival reduced to 0% at a UV-B irradiance of 2.1 J m^{-2} s^{-1} (Marchant et al. 1991).

Regression analysis showed that there was no significant relationship between growth rate after irradiation of the diatoms and the UV-B irradiance they received (Table 3). The growth rate of *Nitzschia lecointei* appeared to decline as UV-B irradiance increased, but, this was not observed until the highest irradiance (3.4 J m^{-2} s^{-1}; Fig. 7). Growth rates for irradiated cultures of *Proboscia alata*, *P. inermis*, *Thalassiosira tumida*, and *Stellarima microtrias* were comparable to those occurring in the PAR control.

Dark-period removal

Removal of the dark period from the irradiance of both *Nitzschia lecointei* and *Stellarima microtrias* elicited a different survival response (Fig. 8) from those treatments incorporating a dark period. *N. lecointei* cells survived an irradiance incorporating dark periods of 1.75 J m^{-2} s^{-1} (Fig. 6A), but survived all but the maximum irradiance when exposed without dark periods (Fig. 8A). In contrast to *N. lecointei*, survival of *S. microtrias* during irradiation including a dark period did not decline significantly until an irradiance of 3.2 J m^{-2} s^{-1}. When the dark period was removed, survival of *S. microtrias* declined to 23% at an irradiance of 1.75 J m^{-2} s^{-1}. In addition, survial of *S. microtrias* over the lower range of UV-B irradiances was ≃ 20% lower in exposures with a dark period (Fig. 6E) than in those without (Fig. 8B).

Discussion

This study was structured to approach natural conditions, so that some insights into the basic responses of diatoms

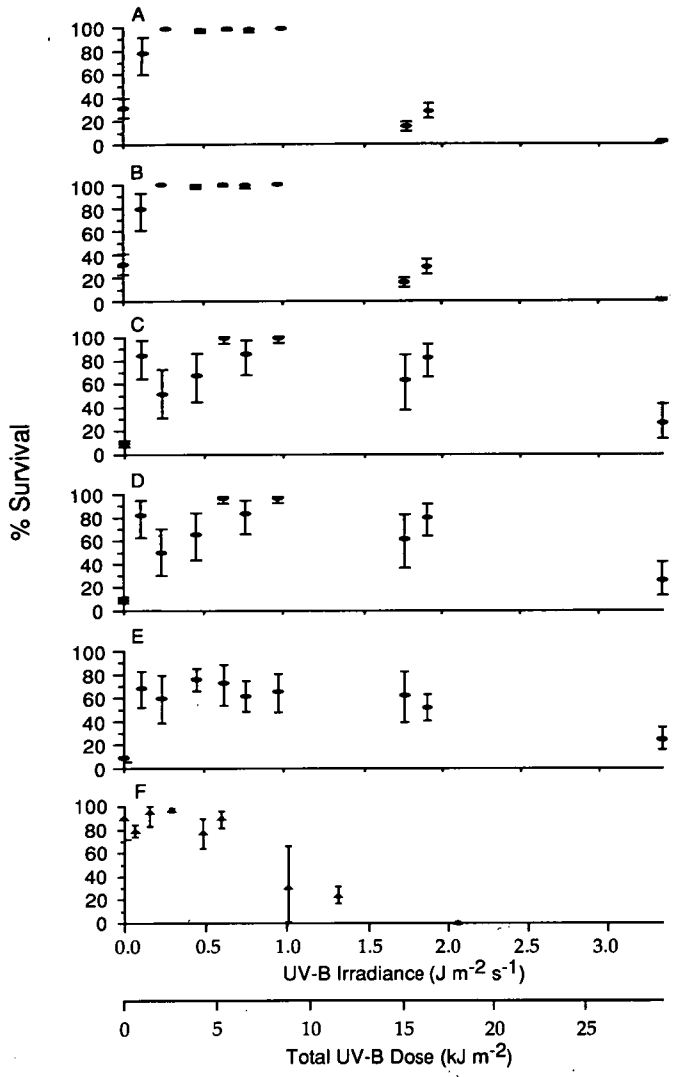


Fig. 6 **A** *Nitzschia lecointei*, **B** *Proboscia alata*, **C** *P. inermis*, **D** *Thalassiosira tumida*, **E** *Stellarima microtrias*, **F** *Phaeocystis* cf. *pouchetti*. Percent survival of diatoms irradiated for 24 h of a 48 h period as a function of UV-B irradiance. (Data for *Phaeocystis* from Marchant et al. 1991). Errors bars represent standard errors calculated from Zar (1984)

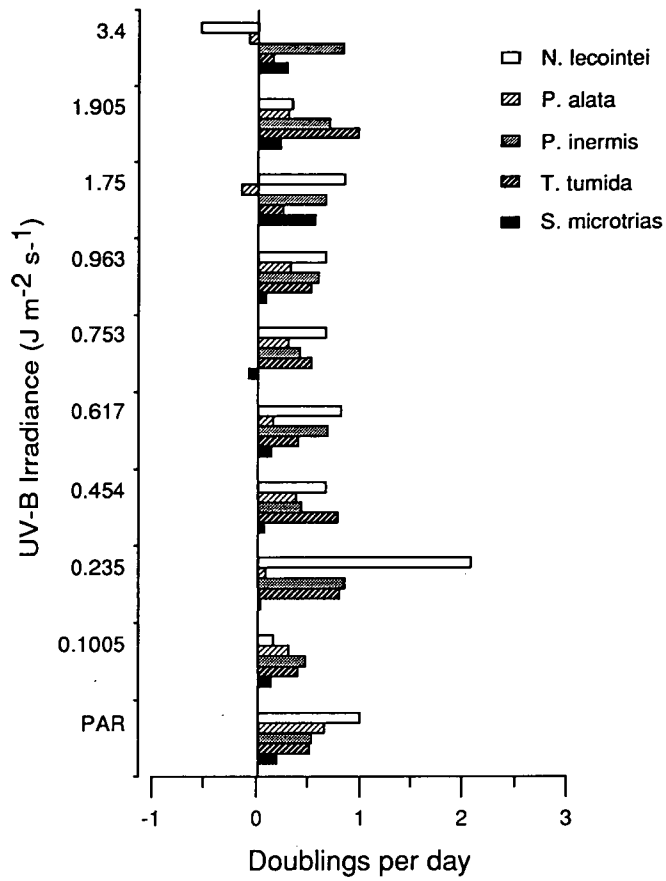


Fig. 7 *Nitzschia lecointei*, *Proboscia alata*, *P. inermis*, *Thalassiosira tumida* and *Stellarima microtrias*. Growth rate of exponentially growing culture after exposure to 12.13±2.13 W m^{-2} (60.5±10.6 µE m^{-2} s^{-1}) PAR, 1.19±0.68 W m^{-2} UV-A, and various UV-B irradiances

Table 3 Regression statistics obtained by linear regression of post-irradiation growth-rate against UV-B irradiance for Antarctic marine diatoms

Species	$P(r)$
Nitzschia lecointei	0.1 <×<0.05
Proboscia alata	0.2 <×<0.1
Proboscia inermis	0.1 <×<0.05
Thalassiosira tumida	0.5 <×<0.2
Stellarima microtrias	0.2 <×<0.1

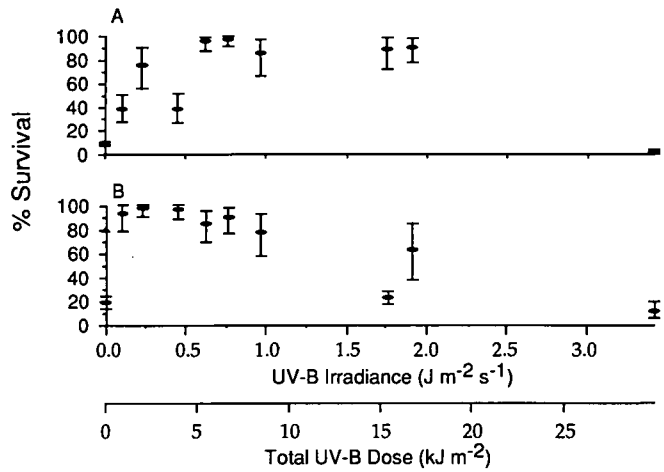


Fig. 8 **A** *Nitzschia lecointei*, **B** *Stellarima microtrias*. Percent survial after 24 h continuous UV-B irradiation. Errors bars represent standard errors calculated from Zar (1984)

to UV-B exposure could be revealed. Experimental limitations included the inability to replicate the dynamic nature of the light climate of the Antarctic marine ecosystem in the laboratory, and the relatively short period over which the UV-B irradiation treatments took place (48 h period with 24 h exposure to UV-B). However, a UV-B exposure of 24 to 48 h reportedly produces significant changes in phytoplankton photosynthetic (Bidigare 1989) and UV-absorbing (Marchant et al. 1991) pigmentation. Numerous Antarctic marine organisms have been shown to produce UV-B-absorbing compounds (Mitchell et al. 1989; Vernet et al. 1989; Karentz et al. 1991 b; Marchant et al. 1991), and there is now a substantial literature indicating that such compounds, principally mycosporine-like amino acids (MAAs), can provide protection against UV-B damage (e. g. Vernet et al. 1989; Carreto et al. 1990; Karentz et al. 1991 b). Marchant et al. (1991) reported a high concentration of UV-B-absorbing compounds in the colonial stage of the Antarctic prymnesiophyte *Phaeocystis*, and demonstrated that the colonial stage of this alga survived higher levels of UV-B irradiation than either the motile stage, or the colonial stage of temperate strains which lacked or contained much lower concentrations of these compounds.

Only compounds with absorption at wavelengths of >290 nm were considered in this study, since shorter wavelengths are not encountered in the marine environment (Smith and Baker 1979; Baker et al. 1980) and would be of no ecological significance in UV protection. The diatoms we investigated have compounds that absorb at UV-A wavelengths, with only low absorbance at the shoulders of these peaks at UV-B wavelengths. The concentrations of UV-absorbing compounds in the diatoms were ≈2 to 5 orders of magnitude less per unit cell C than concentrations in *Phaeocystis*, and – unlike *Phaeocystis* – the concentration of UV-absorbing compounds did not increase significantly as irradiance increased. Therefore, it appears that these diatoms are not using pigments as protection from UV-B to the same extent as does *Phaeocystis*. It remains possible that absorbance at these wavelengths is an incidental consequence of possessing certain cell proteins or metabolites which constitute a target rather than a protective mechanism. Thus, the significance of UV-absorbing compounds in the diatoms as a screen remains uncertain but appears low.

On the basis of the high concentrations of UV-absorbing compounds in *Phaeocystis* and their relative absence in diatoms, Marchant and Davidson (1991) proposed the possibility of a change in the species composition of Antarctic phytoplankton to favour *Phaeocystis* at the expense of diatoms in the marginal ice edge zone. However, the data of the present study demonstrate that diatoms are capable of surviving higher levels of UV-B exposure than *Phaeocystis*. Smith et al. (1992) also found that the rate of cell division of another diatom (*Chaetoceros socialis*) in the Southern Ocean was less affected by a given solar irradiance (including UV-B) than was *Phaeocystis*.

The reports of substantial levels of UV-B-absorbing compounds in mixed phytoplankton from Antarctic waters by Mitchell et al. (1989), Vernet et al. (1989), and Gieskes and Kraay (1990) do not conflict with our data. Their unidentified samples could have contained *Phaeocystis*, an abundant component of the Antarctic phytoplankton community which produces high concentrations of such compounds.

With the exception of *Thalassiosira tumida*, absorption of UV-B by cell concentrations from which MeTHF-soluble pigments had been extracted was considerably greater than the maximum UV absorption by MeTHF-soluble pigments. Absorption of UV-B by the frustule was similar to or greater than absorption by the MeTHF-soluble pigments. Most of the UV-B absorbance in all species except *T. tumida* was due to oxidisable cell contents. The location of these absorbing compounds and structures such as membranes, proteins and carbohydrates within the cell in relation to UV targets within the cell would determine their value as an intracellular screen against UV-B damage. The low absorption by MeTHF-soluble pigments supports the argument that they are not primarily UV-B-protective compounds. Further, the UV-B irradiance at which each species showed a significant decrease in survival did not correlate with their absorption by MeTHF-soluble pigments, frustules or MeTHF-insoluble cell contents. This suggests that none of these fractions provide significant protection from UV-B radiation and that processes other than UV-B screening are responsible for the survival of diatoms at elevated UV-B irradiances.

The pycnocline in the MIZ may be 10 m or less for periods of up to 6 d (Veth 1991). Thus, phytoplankton in this environment may receive high UV-B irradiances for prolonged periods. Our results indicate that diatoms and *Phaeocystis* are able to survive and grow at UV-B irradiances approximately twice (and in the case of *Proboscia inermis* and *Stellarima microtrias* over three times) the peak surface irradiance currently experienced in Antarctic waters for at least 24 h. Their capacity to withstand UV exposure may reflect changes in species composition or selection of UV-resistant strains over the 15 yr of known existence of ozone depletion. Alternatively, high UV-B environments may have existed for substantial periods in their evolution, thus pre-adapting these organisms (Yentsch and Yentsch 1982). Smith et al. (1992) found that the growth rates of phytoplankton after irradiation were independent of the depth from which the samples had been taken, and depended only on the dose received at the depth of incubation. This evidence lends further weight to the idea that these organisms are pre-adapted to a relatively high UV-B environment. Our data also suggests that the impact upon diatoms of increased UV-B irradiance as a result of ozone depletion may be minimal.

The survival of *Thalassiosira tumida* and *Stellarima microtrias* was <100% at UV-B irradiances between 0 and 1.75 J m^{-2} s^{-1}. However, their survival at these irradiances did not appear to be correlated with UV-B irradiance, and *S. microtrias* did not show any significant decline in survival until the highest UV-B irradiance. Thus, the lower maximum survival probably reflects subculturing disturbance or overestimation of the Time 0 population of the PAR-irradiated control culture. The low survival observed

in all species under Mylar screens, which received no UV-B but unattenuated UV-A, indicates that UV-A is also potentially lethal to phytoplankton but that this damage is ameliorated by UV-B. While photorepair of UV-B-induced damage has been reported (Harm 1980; Karentz 1988; Karentz et al. 1991 a), UV-B-facilitated repair of UV-A-induced damage has not. UV-A is largely responsible for the inhibition of carbon fixation (Bühlmann et al. 1987), but it would appear unlikely that photoinhibition alone could be responsible for the observed mortality in Mylar-screened treatments. The short wavelength UV-A emitted from the UV lamps used in these experiments may have been affecting other cellular processes or constituents.

Ongrowth of cultures irradiated over 24 to 48 h indicated that the growth rate of most species was unaffected by the UV-B irradiance to which they were subjected. This was observed even at irradiances which resulted in high mortality. The results indicate that cells which survived sustained their metabolism during irradiation and were then able to resume normal growth, making their growth rate indistinguishable from both control cultures and those cultures which had received much lower UV-B irradiances. Thus, their contribution to the population after irradiance is dependent upon their survival rather than on any persisting consequence of the UV-B dose received.

Estimates of mixing times from the surface layer to a depth of 10 m range from 30 min to hundreds of hours (Denman and Gargett 1983; Karentz 1991). Because of the stability of the MIZ, phytoplankton are subjected to little darkness during late spring and summer (Sakshaug and Skjoldal 1989; Lizotte and Sullivan 1991; Veth 1991). Phytoplankton above the pycnocline will be exposed to changes in UV-B irradiances over periods greater those used in our experiments (24 h). Therefore, these experimental results can only be considered as indicative of natural conditions. The two diatom species that received continuous irradiation at various UV-B irradiances for a 24 h period showed a high tolerance to such exposure. Dark-dependent DNA repair-processes have been cited as fundamentally important to many organisms (prokaryotes, plants and animals) for repair of UV-B induced damage (Harm 1980). Over the 24 h duration of this experiment they appeared to play little part in the survival and reproduction of *Nitzschia lecointei* and *Stellarima microtrias* denied a dark period.

Karentz et al. (1991 a) found that smaller cells with a greater surface area:volume ratio were more sensitive to UV than larger cells. Our results are not consistent with this proposal. The survival of smaller species such as *Nitzschia lecointei* with a surface area to volume ratio of ~ 0.94 was equivalent to that of *Proboscia inermis* with a surface area to volume ratio of ~ 0.20. The observations of Karentz et al. (1991 a) may have been due to cell size and volume affecting sinking rates, which in turn would have affected their relative exposure to UV-B (Denman and Gargett 1983, Karentz 1991, Thompson et al. 1991, Veth 1991).

Conclusions

Our results show that over a 24 to 48 h period, at least some diatoms tolerate levels of UV-B that are considerably higher than the irradiances received in Antarctic surface waters in the austral spring of 1989. The amount of UV-absorbing compounds in the diatom species investigated was much less than that observed in the prymnesiophyte *Phaeocystis*, but their UV-B tolerance exceeds that of *Phaeocystis*. Thus, in constrast to the previously proposed scenario of Marchant and Davidson (1991), any pronounced increase in Antarctic UV-B levels may favour diatoms at the expense of *Phaeocystis*. There is, however, little direct evidence of changes in species composition in the Southern Ocean. The high tolerance of UV-B radiation by the phytoplankton species we have studied suggests that major changes in phytoplankton species composition as a result of extensive UV-B induced mortality are unlikely.

Acknowledgement We gratefully acknowledge Dr. S. Jeffrey for her comments on the manuscript.

References

Baker KS, Smith RC, Green AES (1980) Middle ultraviolet radiation reaching the ocean surface. Photochem Photobiol 32: 367–374

Bidigare RR (1989) Potential effects of UV-B radiation on marine organisms of the Southern Ocean: distributions of phytoplankton and krill during austral spring. Photochem Photobiol 50: 469–477

Bühlmann B, Bossard P, Uehlinger U (1987) The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J Plankton Res 9: 935–943

Calkins J, Thordardottir T (1980) The ecological significance of solar UV radiation on aquatic organisms. Nature, Lond 283: 563–566

Carreto JI, Carignan MO, Daleo G, De Marco SG (1990) Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J Plankton Res 12: 909–921

Denman KL, Gargett AE (1983) Time and space scales of vertical mixing and advection of phytoplankton in the upper ocean. Limnol Oceanogr 28: 801–815

El-Sayed SZ, Stephens FC, Bidigare RR, Ondrusek ME (1990) Effect of ultraviolet radiation on Antarctic marine phytoplankton. In: Kerry KR, Hempel G (eds) Antarctic ecosystems. Ecological change and conservation. Springer-Verlag, Berlin, p 379–385

Eppley RW, Ried FMH, Strickland JDH (1970) Estimates of phytoplankton crop size, growth rate and primary production. In: Strickland JDH (ed) The ecology of the phytoplankton of La Jolla, California in the period April through September, 1967. Bull Scripps Instn Oceanogr 17: 33–42

Gala WR, Giesy JP (1991) Effects of ultraviolet radiation on the primary production of natural phytoplankton assemblages in Lake Michigan. Ecotoxicol Envir Saf 22: 345–361

Gieskes WWC, Kraay GW (1990) Transmission of ultraviolet light in the Weddell Sea: report of the first measurements made in the Antarctic. BIOMASS Newsl (College Station, Tex) 12: 12–14

Guillard RRL, Ryther JH (1962) Studies of the marine plankton diatoms. 1. *Cyclotella nana* Hustedt and *Detonula confervaceae* (Cleve) Gran. Can J Microbiol 8: 229–239

Häder DP, Worrest RC (1991) Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. Photochem Photobiol 53: 717–725

Harm W (1980) Biological effects of ultraviolet radiation. Cambridge University Press, Cambridge

Hobson LA, Hartley FA (1983) Ultraviolet irradiance and primary production in a Vancouver Island fjord, British Columbia, Canada. J Plankton Res 5: 325–331

Karentz D (1988) DNA repair mechanisms in Antarctic marine microorganisms. Antarctic J US 23: 114–115

Karentz D (1991) Ecological considerations of Antarctic ozone depletion. Antarctic Sci 3: 3–11

Karentz D, Cleaver JE, Mitchell DL (1991 a) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. J Phycol 27: 326–341

Karentz D, Lutze LH (1990) Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol Oceanogr 35: 549–561

Karentz D, McEuen FS, Land MC, Dunlap WC (1991 b) Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108: 157–166

Lizotte MP, Sullivan CW (1991) Rates of photoadaptation in sea ice diatoms from McMurdo Sound, Antarctica. J Phycol 27: 367–373

Marchant HJ, Davidson AT (1991) Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. In: Weller G, Wilson CL, Severin BAB (eds) Proceedings of the International Conference on the Role of Polar Regions in Global Change. Geophysical Institute, Fairbanks, Alaska, p 397–400

Marchant HJ, Davidson AT, Kelly GJ (1991) UV-B protecting pigments in the marine alga *Phaeocystis pouchetii* from Antarctica. Mar Biol 109: 391–395

Mitchell BG, Vernet M, Holm-Hansen O (1989) Ultraviolet light attenuation in Antarctic waters in relation to particulate absorption and photosynthesis. Antarctic J US 24: 179–181

Sakshaug E, Skjoldal HR (1989) Life at the ice edge. Ambio 18: 60–67

Smith RC, Baker KS (1979) Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem Photobiol 29: 311–323

Smith RC, Prézelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters, Science, NY 255: 952–959

Smith WO Jr, Nelson DM (1986) Importance of ice edge phytoplankton production in the Southern Ocean. BioSci 36: 251–257

Thompson PA, Harrison PJ, Parslow JS (1991) Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J Phycol 27: 351–360

Vernet M, Mitchell BG, Holm-Hansen O (1989) Ultraviolet radiation in Antarctic waters: response of phytoplankton pigments. Antarctic J US 24: 181–183

Verity PG, Villareal TA, Smayda TJ (1988) Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. 1. Abundance, biochemical composition and metabolic rates. J Plankton Res 10: 219–248

Veth C (1991) The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia–Weddell Sea. J mar Syst 2: 451–464

Vosjan JH, Döhler G, Nieuwland G (1990) Effect of UV-B irradiance on the ATP content of microorganisms of the Weddell Sea (Antarctica). Neth J Sea Res 25: 391–393

Voytek MA (1990) Addressing the biological effects of decreased ozone on the Antarctic environment. Ambio 19: 52–61

Worrest RC, Thomsen BE, Van Dyke H (1981) Impact of UV-B radiation upon estuarine microcosms. Photochem Photobiol 33: 861–867

Yentsch CS, Yentsch CM (1982) The attenuation of light by marine phytoplankton with special reference to the absorption of near-UV radiation. In: Calkins J (ed) The role of solar ultraviolet radiation in marine ecosystems. Plenum, New York, p 691–706

Zar JH (1984) Biostatistical analysis. 2nd ed. Prentice Hall, Englewood Cliffs, New Jersey

Vol. 10: 299-305, 1996 | AQUATIC MICROBIAL ECOLOGY Aquat Microb Ecol | Published June 27

Natural UVB exposure changes the species composition of Antarctic phytoplankton in mixed culture

A. T. Davidson[1,2,*], H. J. Marchant[1], W. K. de la Mare[1]

[1]Australian Antarctic Division, Channel Highway, Kingston, Tasmania, 7050 Australia
[2]Institute of Antarctic and Southern Ocean Studies, University of Tasmania, PO Box 252c, Hobart, Tasmania, 7001 Australia

ABSTRACT: Numerous investigators have demonstrated marked interspecific differences in tolerance of Antarctic marine phytoplankton to UVB exposure. Consequent changes in species composition have been proposed but as yet not demonstrated. We conducted competition experiments in which mixed cultures of Antarctic marine diatoms and colonial and flagellate stages in the life cycle of the haptophyte *Phaeocystis antarctica* were exposed to natural Antarctic solar irradiance. Results demonstrated UVB-induced changes in species composition favouring the colonial stage of *P. antarctica* following 2 d exposure or less. These data indicate the potential for altered trophodynamics and carbon flux in Antarctic waters as a result of ozone depletion. Our results also show the limited predictive value of results obtained using UVB exposures above those likely to be experienced in the natural environment.

KEY WORDS: UVB radiation · Antarctic phytoplankton · Species composition

INTRODUCTION

Stratospheric ozone concentrations over Antarctica presently fall to less than 30% of pre-ozone-hole values during spring (Weiler & Penhale 1994). Springtime UVB irradiance is at least as high as that at the summer solstice (Lubin et al. 1989). Melting sea-ice forms a shallow pycnocline in the marginal ice zone (MIZ) which may confine phytoplankton to depths of 20 m or less for up to 6 d (Mitchell & Holm-Hansen 1991, Veth 1991). Phytoplankton blooms in the high light, high nutrient environment of the MIZ contribute 25 to 67% of phytoplanktonic production in the Southern Ocean (Smith & Nelson 1986).

Survival, growth and photosynthesis of phytoplankton in the MIZ are reduced by UVB exposure (Smith et al. 1992) as they coincide with the springtime ozone depletion (Helbling et al. 1994). It has been suggested that exposure of phytoplankton to increased UVB radiation is likely to alter species composition (Calkins & Thordardottir 1980, Karentz 1991, Smith et al. 1992, Davidson et al. 1994). Such changes have been reported from experimental microcosms in temperate environments using artificially increased UVB irradiance (Worrest et al. 1978, 1981). Interspecific differences in the tolerance of Antarctic phytoplankton to UVB have been reported (Karentz et al. 1991a, Marchant et al. 1991). However, direct evidence of changes in species composition has not been reported for the Southern Ocean.

Species specific investigations of the tolerance of phytoplankton to UVB irradiance are important in predicting the effect of ozone depletion (Karentz 1991, Davidson et al. 1994). However, such studies do not include the competitive interactions between phytoplankton species during UVB exposure. Here we report that exposure of mixed cultures of Antarctic phytoplankton to natural and attenuated Antarctic UV irradiance produced changes in phytoplankton species composition.

MATERIALS AND METHODS

Unialgal strains of the diatoms *Chaetoceros simplex* Ostenfeld, *Fragilariopsis lecointei* V. H., *Fragilariopsis*

*E-mail: andrew_dav@antdiv.gov.au

curta (V. H.) Hasle, *Thalassiosira tumida* (Jan.) Hasle, *Proboscia (Rhizosolenia) alata* (Brightwell) Sundström and the haptophyte *Phaeocystis antarctica* Karsten were isolated from Prydz Bay, Antarctica. Cultures were maintained under cool white fluorescent light at a photosynthetically active radiation (PAR) intensity of 5.11 W m^{-2} and at 0°C with an 18:6 h light:dark cycle. Exponentially growing cultures of the 6 species (that of *P. antarctica* containing approximately equal concentrations of the flagellate and colonial life stages) were diluted 1:5, culture:fresh culture medium, 5 and 2 d before starting the experiment. Organisms in 10 ml subsamples of each monospecific culture were fixed with buffered Lugol's solution and the cell concentration estimated using the Utermöhl sedimentation technique over 15 replicate randomly chosen fields using an inverted microscope. Aliquots of each culture were mixed to give approximately equal cell concentrations of each species and both *P. antarctica* life stages. Three 10 ml subsamples were removed to determine cell concentrations (as above) of each species at time 0. Nine subsamples, each of 500 ml, were then transferred to polythene bags (WhirlPak, Nasco) which transmit light above 220 nm. Three replicate bags were each exposed to one of 3 light treatments; unscreened (PAR, UVA and UVB treatment), Mylar screened (which transmitted wavelengths above 320 nm: PAR + UVA treatment), and polycarbonate screened (which transmitted wavelengths above 370 nm: PAR treatment) (Davidson & Marchant 1994).

Mixed phytoplankton populations were incubated for 8 d at Davis Station, Antarctica (68° 35' S, 78° E) at a depth of 0.2 m between 10 and 18 December 1992 in an outdoor tank through which sea water was circulated. Thus, the phytoplankton were exposed to near-surface natural light irradiance. Integrated irradiances were measured using an IL 1700 research radiometer equipped with UVA and erythemal UVB sensors (Davidson & Marchant 1994) which were calibrated to solar irradiances using the sensor response curve and a Macam spectroradiometer and erythemal UVB biometer respectively. Sensors were positioned beside phytoplankton at 0.2 m depth and the UVA and UVB irradiance integrated during incubation.

A subsample of 10 ml was removed from each replicate treatment at 2 d intervals for 8 d and the concentration of each species determined (as above). The cell concentrations of each species after each period of irradiation were used to estimate a single exponential growth rate for each replicate. This provided independent estimates of growth rate, with estimated variances for each species under each of the 3 UV treatments. Exponential ($\log_e$) growth rate estimates were obtained as the slope parameters of a generalised linear model (GLM) (see Chambers & Hastie 1993), using the S-Plus statistical package with Gaussian errors, a log-link function, and weighted by the inverse of the square of the empirical standard error for each cell concentration determination.

The growth rate of cells of each species in the culture became the dependent variable in a fully crossed 2-way analysis of deviance (similar to an ANOVA, but allowing the inverse variances of the estimated growth rates to be used as weights) using a GLM with a Gaussian error model. Thus, growth rates of low variance received higher weight in statistical analysis than those with high variance. Growth rates across all species and by each species were compared between light treatments and presented as a box and whisker plot and interaction profile (see Fig. 1A, B). The flagellate and colonial life stages of *Phaeocystis antarctica* were considered to be functionally separate taxa due to the widely accepted physiological differences between these stages (e.g. Marchant et al. 1991, Davidson & Marchant 1992b).

The size of 100 live cells of each species was measured and the mean cell volume calculated. Variation in the dimensions of cells fixed with Lugol's iodine from each light treatment were within 1 standard deviation of the live cell dimensions. The cell concentrations in replicates of each light treatment at each incubation time were pooled and the mean and standard error computed. Using the equations of Eppley et al. (1970) and the cell volume, carbon contributed by each species was then calculated. The carbon contributed by colonial stage *Phaeocystis antarctica* was likely to be an underestimate as colony matrix was not considered in the calculation.

To investigate the minimum duration of UV exposure required to cause changes in phytoplankton species composition, a 10 ml subsample was removed from each replicate of all light treatments after 2, 4, 6 and 8 d incubation and inoculated into 40 ml of sterile *f*/2 medium in 50 ml polystyrene culture flasks. Flasks were returned to culture maintenance conditions and grown for a further 9 d then thoroughly mixed and a 10 ml subsample removed and counted (as above). This procedure of subculturing samples after the various durations of exposure allowed expression and amplification of changes in phytoplankton species composition, while avoiding the effects of nutrient limitation in culture. For each incubation time and light treatment, the mean proportion of the phytoplankton population contributed by each species following the 9 d amplification was calculated over 3 replicates. For these proportional data, error bars indicate ±1 standard error, calculated by arcsine square root transformation (after Zar 1984).

Table 1. Cumulative integrated UVA and UVB irradiance during 8 d natural irradiation measured using an International Light research radiometer and light sensors

Irradiation (d)	Cum. integr. UVA (J m^{-2})	Cum. integr. erythemal UVB (J m^{-2})
0	0	0
2	9.75×10^5	6.48×10^3
4	18.43×10^5	12.26×10^3
6	28.51×10^5	19.74×10^3
8	36.59×10^5	25.18×10^3

RESULTS AND DISCUSSION

Weather conditions were intermittently sunny during the 8 d of exposure to natural Antarctic solar radiation. However, integrated UVA and UVB irradiances varied little during between-sample intervals (Table 1). Mean integrated UVA and UVB irradiance for each 2 d incubation period was $9.15 \pm 0.93 \times 10^5$ and $6.30 \pm 0.90 \times 10^3$ J m^{-2} respectively.

The analysis of deviance shows significant differences with species, light treatment and their interaction (Table 2). That species specific growth rates are significantly different is not surprising. The distributions of growth rates across species for the different UV treatments are shown in Fig. 1A. Although the main effect due to UV treatment is significant (Table 2), the effects are not substantial, and the statistical significance arises largely because of a few cases where growth rate estimates with low variances have received a high weight in the analysis. The unweighted means and medians of the growth rates across species are not significantly different between light treatments. Similarly, no significant difference was found in total calculated cell carbon concentration between light treatments (Fig. 2A). Thus, the overall growth and production by the community was maintained irrespective of light treatment.

In contrast, the interaction term is highly significant (Table 2). The changes in growth rates for at least some species under the different UV treatments demonstrates that, over time, the species composition, in terms of cell concentration, will differ under the different UV regimes. Differences between the PAR and PAR + UVA light treatments, though significant [$p(\chi^2 < 0.01)$], were only slight. Exposure to UVB caused substantial changes in the growth rates (Fig. 1B) which were statistically highly significant [$p(\chi^2 < 0.000005)$].

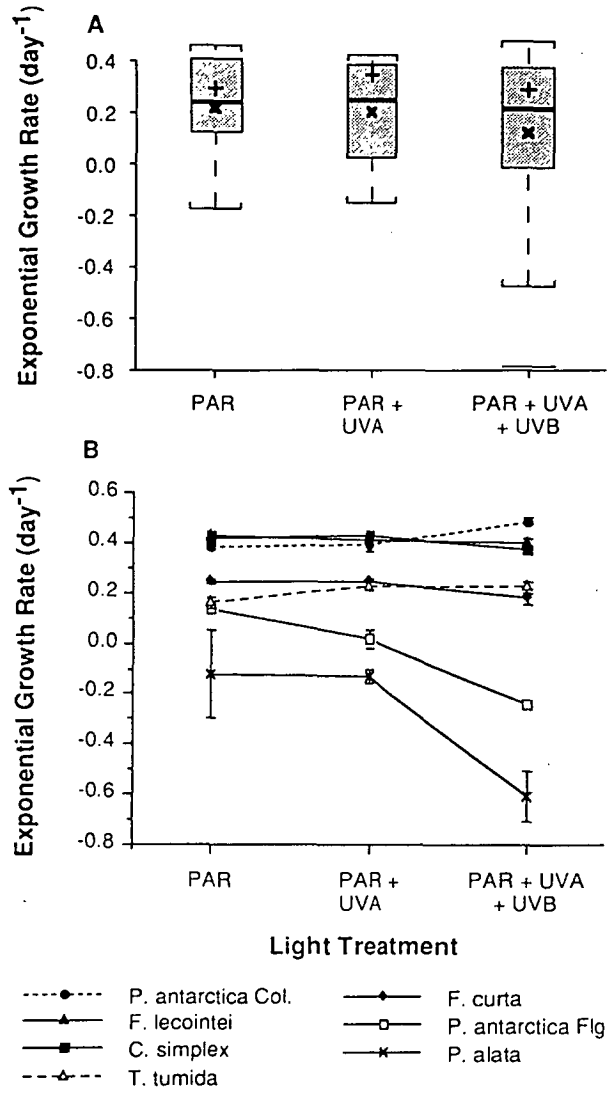


Fig. 1. Analysis of exponential growth rate (d^{-1}) during 8 d exposure to natural irradiation. (A) Box and whisker plot showing similar growth rate across all species and comparing between light treatments and (B) interaction profile showing UVB-dependent changes in the growth rate of each species between light treatments with the standard error over 3 replicates. Box and whisker plot shows mean weighted (+), unweighted (x) and median (–) growth rates for all species between light treatments. Boxes enclose the interquartile range, whiskers extending to the standardised range and '—' represents an outlier

Table 2. Two-way analysis of deviance showing the significance of UV effects on the growth of Antarctic phytoplankton species

Factor	df	Deviance	Residual df	Residual deviance	Probability χ^2
Null			62	2809.445	
Species	6	2620.609	56	188.836	0.00000000
UV treatment	2	11.630	54	177.206	0.00298
Species:UV interaction	12	134.048	42	43.158	0.00000000

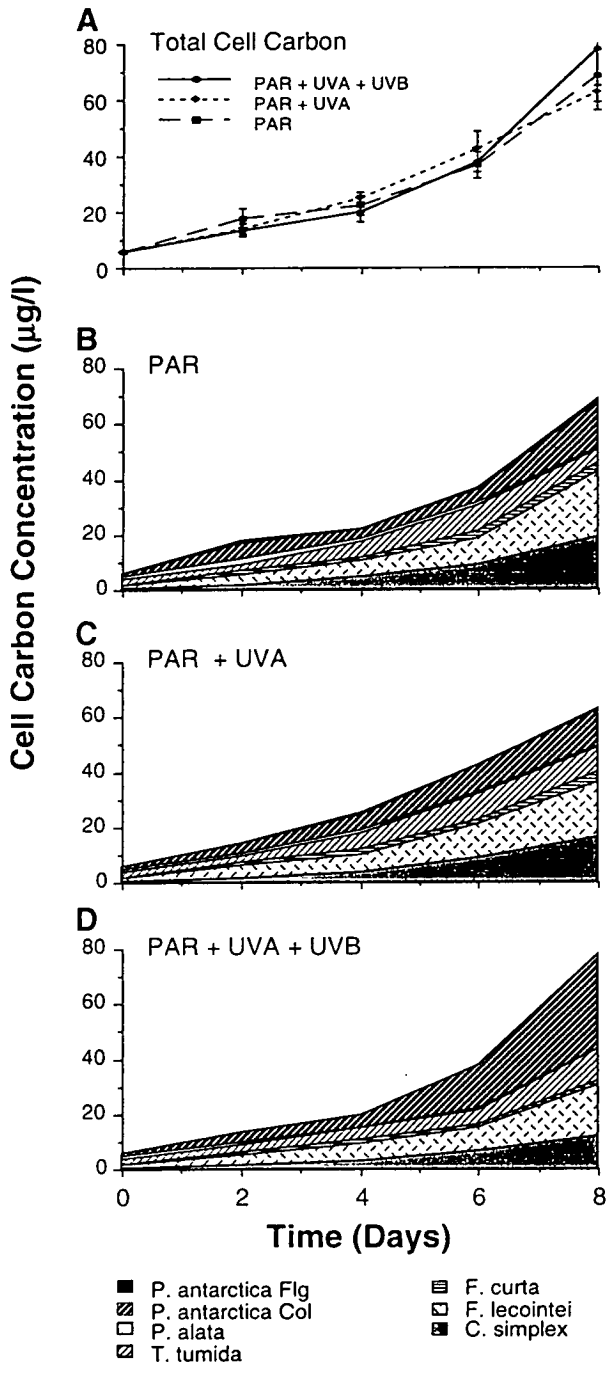


Fig. 2. Calculated cell carbon concentration of Antarctic phytoplankton during 8 d of natural irradiation showing (A) total cell carbon across all species, and contributions by each species exposed to (B) PAR, (C) PAR + UVA and (D) PAR + UVA + UVB. *P. antarctica* Col and Flg indicate the colonial and flagellate life stage respectively. Error bars indicate standard error of the mean over 3 replicates

The growth rate of diatoms and the flagellate stage of *Phaeocystis antarctica* exposed to UVB either did not change significantly or declined. Only the colonial stage of *P. antarctica* showed a substantial promotion of growth as a result of exposure to UVB. The proportion of cell carbon contributed by diatoms and the flagellate stage of *P. antarctica* exposed to UVB also fell (Fig. 2B–D) but were replaced by colonial stage *P. antarctica*. Thus, Antarctic near-surface UVB irradiance alters phytoplankton species composition in culture.

The enhanced growth of colonial *Phaeocystis antarctica* when exposed to UVB agrees with earlier findings which demonstrated that exposure of unialgal cultures of this species to *in situ* UVB increased cell size, growth rate and production by the colonial stage (Davidson & Marchant 1994) and may contribute to this species being one of the first to bloom in the ice and surface waters, where it frequently dominates the phytoplankton (Garrison et al. 1987, Fryxell & Kendrick 1988, Davidson & Marchant 1992a). Only *Proboscia alata* exhibited no significant growth in the absence of UV radiation (Fig. 1B). This may reflect the observed sensitivity of the species to mechanical disturbance during subculturing which leads to an extended lag phase in its growth.

Our results differ from those reported by other authors. McMinn et al. (1994) found that sediment cores from fjords in East Antarctica did not exhibit evidence of a significant change in species composition of the diatom community since springtime ozone depletion began. However, it is likely that persistent sea ice, which attenuates UVB by at least 90% (Trodahl & Buckley 1989), provided shielding for these organisms. Bothwell et al. (1995) also criticise other aspects of the conclusions drawn by McMinn et al. (1994). Others (Smith et al. 1992, Karentz 1994, Karentz & Spero 1995) have reported that exposure of diatoms and *Phaeocystis antarctica* to natural UVB did not alter their growth rates or that growth by *P. antarctica* declined.

Our study used natural solar irradiance and selected Antarctic phytoplankton species but did not simulate natural Antarctic conditions. We used a limited species assemblage grown in nutrient enriched media with and without exposure to a near-surface UVB light climate and wavelength structure. The effects of *in situ* UVB radiation on naturally occurring phytoplankton communities could differ from those we observed and the effect of increased UVB flux as a result of ozone depletion on phytoplankton species composition remains to be ascertained. Results obtained by Karentz (1994) for *Phaeocystis antarctica* were highly variable due to the clumped distribution of cells in colonies, changes within or between light treatments were seldom significant and, unlike Smith et al. (1992) and Davidson & Marchant (1994), *P. antarctica* growth was also negative irrespective of light treatment. Differences between the results presented here and those of Smith et al. (1992) may be due to differences in

methodology, the physiological state of cells or our use of cultured material and a multi-species mix. Karentz & Spero (1995) report a strong positive correlation between *P. antarctica* concentration and column ozone concentration in the MIZ of the Bellingshausen Sea. The apparent conflict between their results and those presented here can only be reconciled with further study. Differences in methodology mean the studies are not directly comparable and it remains unclear whether the changes in *P. antarctica* concentration observed by Karentz & Spero (1995) were directly related to changes in the *in situ* UVB climate during their study.

Our results also contrasted with our previous findings. The colonial stage in the life cycle of *Phaeocystis antarctica* produces high concentrations of UV-absorbing compounds (Marchant et al. 1991). These enhanced survival of its colonial stage when exposed to high UVB irradiances (Marchant et al. 1991) but diatoms, which largely lack UV absorbing compounds, survived UVB irradiances 3 to 5 times that which caused mortality in colonial *P. antarctica* (Davidson et al. 1994). Thus, the role of UV-absorbing compounds in alleviating UVB damage is questionable. Many Antarctic marine organisms possess UV-absorbing compounds (Karentz et al. 1991b). However, the presumed protection afforded organisms by such compounds remains largely unquantified. Results presented here show that growth by the colonial stage of *P. antarctica* was promoted under natural UVB exposure. Consequently, the UVB irradiance at which *P. antarctica* died (Marchant et al. 1991) was not indicative of its enhanced growth at sub-lethal natural irradiances. Nor was survival of diatoms up to far higher UVB irradiances than *P. antarctica* (Davidson et al. 1994) indicative of their slowed growth and production at these sub-lethal irradiances as here we show that *P. antarctica* dominates at their expense. The poor predictive value of a species response to high UVB irradiance experiments clearly demonstrates the limited value of extrapolating results of such experiments (Worrest et al. 1978, Karentz et al. 1991a, Marchant et al. 1991, Davidson et al. 1994) to the natural environment.

Vernet et al. (1994) found that high haptophyte concentrations in Antarctic waters correlated with high *in situ* absorption at 330 nm and low inhibition of photosynthesis when exposed to UVB. At sublethal natural UVB irradiances, metabolic processes such as photosynthesis apparently are shielded from damage by UV-absorbing compounds. Other metabolic costs of exposure to UVB are thereby minimised. Thus, at natural UVB irradiances colonial *Phaeocystis antarctica* may be afforded substantial protection by UV-absorbing compounds. High *in situ* absorption (Vernet et al. 1994) also suggests that blooms of *P. antarctica* may confer some UV protection on other organisms in the water column (Marchant et al. 1991), a feature not included in this experiment as colonial *P. antarctica* did not reach sufficient concentrations to attenuate UVB throughout the irradiated cultures.

The duration of UVB exposure required to elicit changes in phytoplankton species composition is critical in determining the potential magnitude of changes in phytoplankton species composition in Antarctic waters. Incubations of only 2 to 6 d may not have allowed expression of these changes in species composition. To express and amplify such changes, samples were removed from natural irradiation, subcultured, returned to culture maintenance conditions (which lack UV) and grown for a further 9 d. Differences in proportional abundance of each species were not greatly increased by exposure times exceeding 2 d (Fig. 3). Thus, 2 d exposure to ambient near-surface UVB irradiance was sufficient to largely determine the UVB-mediated species composition. Exposure to UVA and UVA + UVB increased the proportion of colonial *Phaeocystis antarctica* in culture, mainly at the expense of *Chaetoceros simplex* (Fig. 3A, B). The proportion of total cells contributed by other species differed little between light treatments (Fig. 3C–E). Two days was the shortest natural exposure time investigated; the minimum exposure required to elicit changes in species composition remains unknown.

Results demonstrate that natural Antarctic UVB irradiance can alter phytoplankton species composition and also indicate that ozone depletion and the associated increase in UVB may promote the abundance of *Phaeocystis antarctica* relative to diatoms in Antarctic waters. This species accounts for some 10% of the total biogenic flux of dimethylsulfide (DMS) released to the atmosphere (Gibson et al. 1990). DMS is a principal source of sulfate cloud condensation nuclei and influences oceanic cloud cover (Charlson et al. 1987). *P. antarctica* also plays a pivotal role in determining the structure and function of the planktonic community (Garrison et al. 1987, Fryxell & Kendrick 1988, Davidson & Marchant 1992a). Its blooms produce high concentrations of dissolved organic carbon and slow-sinking mucilaginous particulate organic carbon which promote bacteria and microheterotrophs, but the large mucilaginous colonies of this alga are selectively avoided by many grazers (Davidson & Marchant 1992b). Thus, any UVB-mediated increase in the abundance of *P. antarctica* could effect climate through changes in global albedo (Charlson et al. 1987) and significantly alter rates of vertical carbon flux and the particle size, form and availability of carbon to higher trophic levels (Marchant & Davidson 1991).

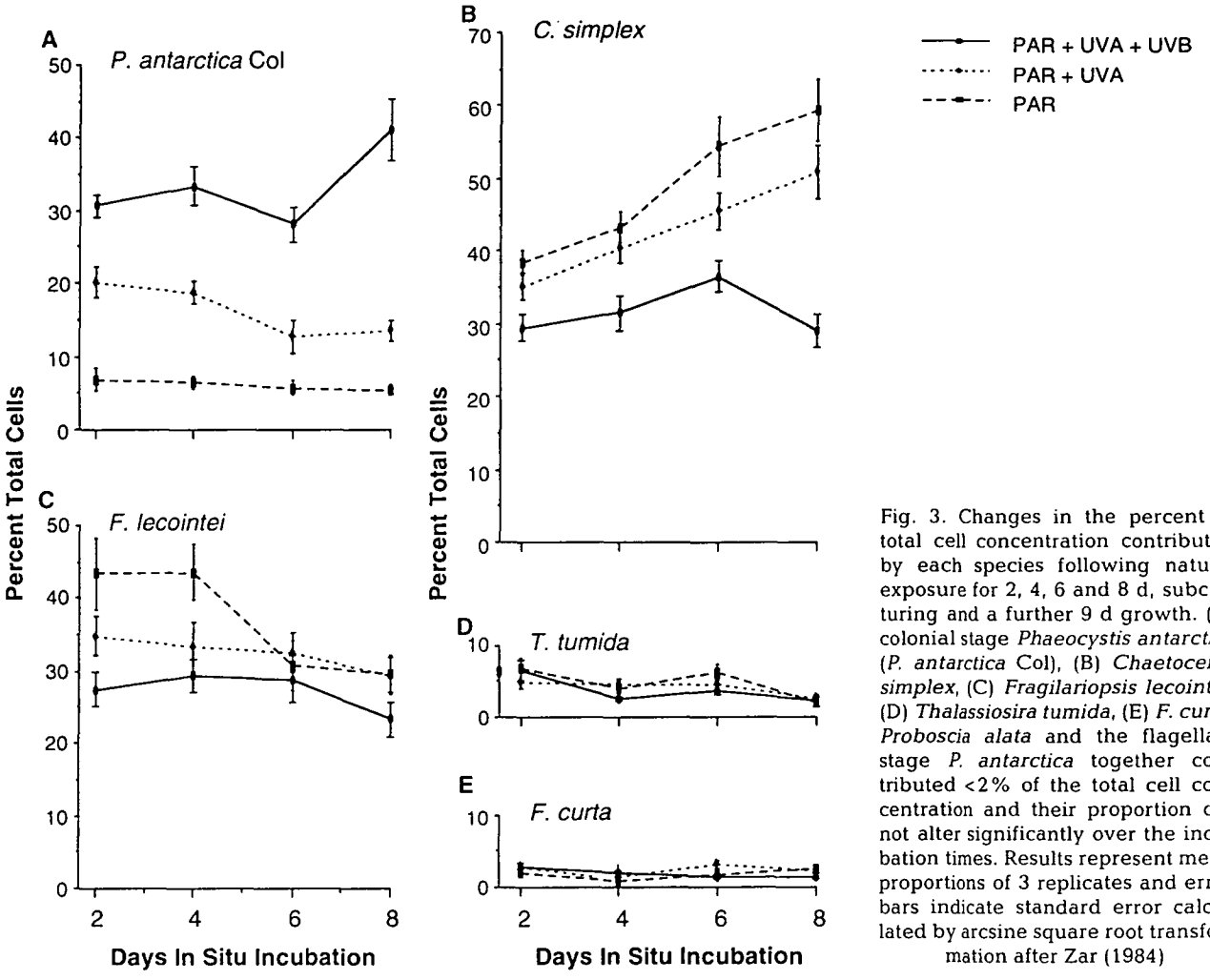


Fig. 3. Changes in the percent of total cell concentration contributed by each species following natural exposure for 2, 4, 6 and 8 d, subculturing and a further 9 d growth. (A) colonial stage *Phaeocystis antarctica* (*P. antarctica* Col), (B) *Chaetoceros simplex*, (C) *Fragilariopsis lecointei*, (D) *Thalassiosira tumida*, (E) *F. curta*. *Proboscia alata* and the flagellate stage *P. antarctica* together contributed <2% of the total cell concentration and their proportion did not alter significantly over the incubation times. Results represent mean proportions of 3 replicates and error bars indicate standard error calculated by arcsine square root transformation after Zar (1984)

LITERATURE CITED

Bothwell ML, Karentz D, Carpenter EJ (1995) No UVB effect? Nature 374:601

Calkins J, Thordardottir T (1980) The ecological significance of solar UV radiation on aquatic organisms. Nature 283: 563–566

Chambers JM, Hastie TJ (1993) Statistical models in S. Chapman and Hall, London

Charlson RJ, Lovelock JE, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature 326:655–661

Davidson AT, Bramich D, Marchant HJ, McMinn A (1994) Effects of UV-B radiation on growth and survival of Antarctic marine diatoms. Mar Biol 119:507–515

Davidson AT, Marchant HJ (1992a) Protist interactions and carbon dynamics of a *Phaeocystis*-dominated bloom at an Antarctic coastal site. Polar Biol 12:387–395

Davidson AT, Marchant HJ (1992b) The biology and ecology of *Phaeocystis* (Prymnesiophyceae). In: Round FE, Chapman DJ (eds) Progress in phycological research, Vol 8. Biopress, Bristol, p 1–45

Davidson AT, Marchant HJ (1994) Comparative impacts of in situ UV exposure on productivity, growth and survival of Antarctic *Phaeocystis* and diatoms. Proc NIPR Symp Polar Biol 7:53–59

Eppley RW, Reid FMH, Strickland JDH (1970) Estimates of phytoplankton crop size, growth rate and primary production. In: Strickland JDH (ed) The ecology of the phytoplankton of La Jolla, California in the period April through September, 1967. Bull Scripps Inst Oceanogr 1:33–42

Fryxell GA, Kendrick GA (1988) Austral spring microalgae across the Weddell Sea ice edge: spatial relations found during a northward transect during AMERIEZ 83. Deep Sea Res 35:1–20

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

Gibson JAE, Garrick RC, Burton HR, McTaggart AR (1990) Dimethylsulfide and the alga *Phaeocystis* in Antarctic coastal waters. Mar Biol 104:339–346

Helbling EW, Villafañe V, Holm-Hansen O (1994) Effects of Ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic Research Series, Vol 62. American Geophysical Union, Washington, DC, p 207–228

Karentz D (1991) Ecological considerations of Antarctic ozone depletion. Antarct Sci 3:3–11

Karentz D (1994) Ultraviolet tolerance mechanisms in Antarctic marine organisms. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic Research Series, Vol 62. American Geophysical Union, Washington, DC, p 93–110

Karentz D, Cleaver JE, Mitchell DL (1991a) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. J Phycol 27: 326–341

Karentz D, McEwan FS, Land KM, Dunlap WC (1991b) A survey of micosporine-like amino acid compounds in Antarctic marine organisms: potential protection from ultraviolet exposure. Mar Biol 108:157–166

Karentz D, Spero HJ (1995) Response of *Phaeocystis* population to ambient fluctuations of UVB radiation caused by Antarctic ozone depletion. J Plankton Res 17 (9): 1771–1789

Lubin D, Frederick JE, Booth CR, Lucas T, Neuschuler D (1989) Measurements of enhanced springtime ultraviolet radiation at Palmer Station, Antarctica. Geophys Res Lett 16:783–785

Marchant HJ, Davidson AT (1991) Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. In: Weller G, Wilson CL, Severin BAB (eds) Proceedings of the International Conference on the Role of Polar Regions in Global Change. Vol 2, Geophysical Institute, Fairbanks, p 397–400

Marchant HJ, Davidson AT, Kelly GJ (1991) UV-B protecting pigments in the marine alga *Phaeocystis pouchetii* from Antarctica. Mar Biol 109:391–395

McMinn A, Heijnis H, Hodgson D (1994) Minimal effects of UVB radiation on Antarctic diatoms over the past 20 years. Nature 370:547–549

Mitchell BG, Holm-Hansen O (1991) Observations and modelling of Antarctic phytoplankton crop in relation to mixing depth. Deep Sea Res 38:981–1007

Smith RC, Prezelin BB, Baker KS, Bidigare RR, Boucher NP, Coley T, Karentz D, MacIntyre S, Matlick HA, Menzies D, Ondrusek M, Wan Z, Waters KJ (1992) Ozone depletion: ultraviolet radiation and phytoplankton biology in Antarctic waters. Science 255:952–959

Smith WO Jr, Nelson DM (1986) Importance of ice edge phytoplankton production in the Southern Ocean. BioSci 36:251–257

Trodahl HJ, Buckley RG (1989) Ultraviolet levels under sea ice during the Antarctic spring. Science 245:194–195

Vernet M, Brody EA, Holm-Hansen O, Mitchell, BG (1994) The response of Antarctic phytoplankton to ultraviolet radiation: absorption, photosynthesis and taxonomic composition. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic Research Series, Vol 62. American Geophysical Union, Washington, DC, p 143–158

Veth C (1991) The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia-Weddell Sea. J Mar Syst 2:451–464

Weiler CS, Penhale PA (1994) Preface. In: Weiler CS, Penhale PA (eds) Ultraviolet radiation in Antarctica: measurements and biological effects. Antarctic Research Series, Vol 62. American Geophysical Union, Washington, DC, p xi–xii

Worrest RC, Thomson BE, Van Dyke H (1981) Impact of UV-B radiation upon estuarine microcosms. Photochem Photobiol 33:861–867

Worrest RC, Van Dyke H, Thomson BE (1978) Impact of enhanced simulated solar ultraviolet radiation upon a marine community. Photochem Photobiol 27:471–478

Zar JH (1984) Biostatistical analysis, 2nd edn. Prentice-Hall, Englewood Cliffs, NJ

Responsible Subject Editor: S. Chisholm, Cambridge, Massachusetts, USA

Manuscript first received: October 27, 1995
Revised version accepted: February 20, 1996

Polar Biol (1992) 12:387 - 395

Polar Biology

Protist abundance and carbon concentration during a *Phaeocystis*-dominated bloom at an Antarctic coastal site

Andrew T. Davidson and Harvey J. Marchant

Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia

Received 4 July 1991; accepted 9 December 1991

Summary. Changes in the concentrations of bacteria, phytoplankton, protozoa, dissolved organic carbon (DOC), particulate organic carbon (POC), particulate carbohydrate (PCHO) and particulate organic nitrogen (PON) were followed throughout the summer at an Antarctic coastal site. The colonial prymnesiophyte *Phaeocystis pouchetii* was the first major phytoplankton species to bloom, reaching concentrations of 6×10^7 cells $\cdot l^{-1}$ and remained numerically dominant for most of the summer. During the *P. pouchetii* bloom the concentration of most other autotrophs did not increase. Microheterotroph abundance peaked during or immediately after the *Phaeocystis* bloom. Their peak coincided with very high concentrations of organic carbon, particularly DOC which exceeded 100 mg $\cdot l^{-1}$, and low bacterial abundance. Maximum bacterial abundance was reached after the decline in microheterotroph numbers. Bacterial utilization of carbon substrates and microheterotroph grazing of bacteria and uptake of DOC may form an important link to higher trophic levels during Antarctic *Phaeocystis* blooms.

Introduction

Numerous studies have been conducted at Antarctic coastal sites and at ice edges to ascertain the species composition and abundance of protists (eg. Buck and Garrison 1983; Garrison et al. 1987; Lipski 1987; Perrin et al. 1987; Fryxell and Kendrick 1988; Garrison and Buck 1989a). However, few reports have addressed the interactions between the various protists, bacteria and available carbon sources. Comparison of our data with previous studies conducted at this coastal site near Davis Station, Antarctica (Perrin et al. 1987; Gibson et al. 1990; Marchant and Perrin 1990) indicate a high level of interannual consistency in bacterial abundance and the dominant phytoplankton and choanoflagellates. Thus, the processes determining community composition at this site repeatedly produce a similar order of succession and abundance of organisms.

Primary production of the Southern Ocean supports substantial bacterial production. Correlation between production by phytoplankton and bacteria has been demonstrated around the Antarctic continent at different times of the year (eg. Fuhrman and Azam 1980; Hanson et al. 1983a, b; Kogure et al. 1986; Billen et al. 1987). However, little attention has been given to changes in the abundance of Antarctic marine bacteria at a single location over time. Kottmeier and Sullivan (1990) suggest that bacterial growth in pack ice may determine concentrations of nutrients and microheterotrophs and show that bacterial production is a significant source of carbon in the ice edge zone. Patterns of bacterial abundance in the water column vary. Satoh et al. (1989) found a single peak in bacterial numbers in December near Syowa station, while Gibson et al. (1990) described a double peak with maxima in November and February near Davis station with the concentration at the end of December being the lowest for any time of the year. Here we report the variation in bacteria, protists and carbon concentrations through the summer in Antarctic coastal waters near Davis and indicate the pivotal role of *Phaeocystis* in determining their abundance in this environment.

Materials and methods

Sampling was conducted in Prydz Bay at a site 5 km offshore from the Australian Antarctic station of Davis (68° 30′ S, 77° 50′ E). Temperature profiles from surface to the bottom depth of 100 m were obtained using a Yeo-Kal Model 606 Submersible Data Logger. A Niskin bottle was used to obtain 10 l water samples at a depth of 15 m at approximately weekly intervals between 15/11/88 and 21/2/89. A depth of 15 m was selected to sample both the shallow ice edge bloom and the protistan community as the pycnocline deepened. All apparatus used for sampling water destined for chemical analysis was soaked in 5% Decon 90 detergent for no less than one week, then soaked in 5% HCl for the same time and thoroughly rinsed in MilliQ or Elgastat deionised water. The contents of the

Correspondence to: H. J. Marchant

Niskin bottle was thoroughly mixed before removing subsamples for counts of bacteria, phytoplankton and chemical analysis. All water samples were handled in a laminar flow hood.

Acid Lugol's solution was used to fix 500 ml of sea-water and the samples allowed to sediment for at least one week in the sample bottles before pipetting off the supernatant. The sedimented concentrates, or subsamples when cell concentrations were high, were then further sedimented for 1 day in 32 mm diameter sedimentation cylinders before cells were counted. Cells greater than 3 μm diameter were counted in fifteen replicate fields of view using an inverted microscope at 200 × magnification and the mean and standard deviation calculated. Glutaraldehyde (4% final concentration) was used to fix 80 ml of the water sample which was then stored in sterile bottles. Subsamples of between 1 and 20 ml, depending on bacterial concentrations, were stained with 4',6-diamidino-2-phenylindole (DAPI) (Porter and Feig 1980), filtered onto 0.1 μm Nuclepore filters and total bacteria (epibacteria plus bacterioplankton) counted by epifluorescent microscopy.

Nonmetric multidimensional scaling (MDS) and cluster analysis were carried out on species abundance data using the methods of Field et al. (1982). Data for those autotrophs and heterotrophs contributing more than 4% of the protist abundance at any one sample time were used. As all data scales were identical, cell concentrations were log transformed ($Y_{ij} = \log(X_{ij} + 1)$) when comparing samples and the concentrations were relativized ($Y_{ij} = 100 \cdot X_{ij} / \Sigma_j = 1^n \cdot X_{ij}$) for the inverse comparison of species. The Bray-Curtis index of similarity was used and the data classified using group average sorting. The MDS starting configuration was generated by principal coordinates analysis and run over 75 iterations.

Water samples of 2.5 l, or until the filter clogged at higher cell densities, were passed through 0.45 μm pore size acid-cleaned Millipore membrane filters. Two 5 ml rinses of 3.6% w/v $(NH_4)_2CO_3$ and one of 1 mM Na_2 EDTA in 3.6% w/v $(NH_4)_2CO_3$ were then passed through the filter. The filter was then sectioned into eighths, and two opposite segments analysed for carbohydrates (CHO) using the phenol sulphuric method (Marshall and Orr 1962). Another 500 ml of water were passed through 25 mm diameter Whatman GF/F filters (particle retention > 0.45 μm) that had been fired at 450°C for 16 h. This filter was sectioned into eight and two of the segments analysed for carbon and nitrogen using a 185B Hewlett Packard Carbon Hydrogen Nitrogen analyser with a 3380A Hewlett Packard Integrator. A catalyst of three parts MnO_2:1 part Cr_2O_3:1 part diatomaceous earth was used to enhance oxidation at the furnace temperature of 800°C with a combustion time of 50 s. Approximately 20 ml of filtrate were stored in teflon capped glass vials at 4°C prior to analysis for DOC. DOC concentration was measured using a Technicon auto Analyzer II sampler, La Jolla PO-24 Photo-oxidation unit and Horiba PIR-2000 infrared gas analyser. Oxidation of DOC to CO_2 by UV light was enhanced by saturation with ultra pure O_2. Ultra pure N_2 was used as the carrier gas to the infrared gas analyser.

Results

Water column characteristics

Sea ice cover was 100% until 3/1/89 after which it declined to approximately 70% cover until 24/1/89 and approximately 50% thereafter (Fig. 1). Ice was completely absent by 1/2/89 but 70% ice cover had reformed by 21/2/89.

The temperature profile (Fig. 1) shows that the water column was unstructured until after 7/12/88. Between 22/12/88 and 17/1/89 surface temperature rose to its maximum of 1.13°C and the depth of warming increased with time. This thermal structuring of the water column occurred despite there being 70%–100% ice cover and indicates advection of water from nearby ice free areas into the sample site. Surface water temperature had begun to fall by the time the ice was absent from the sampling site.

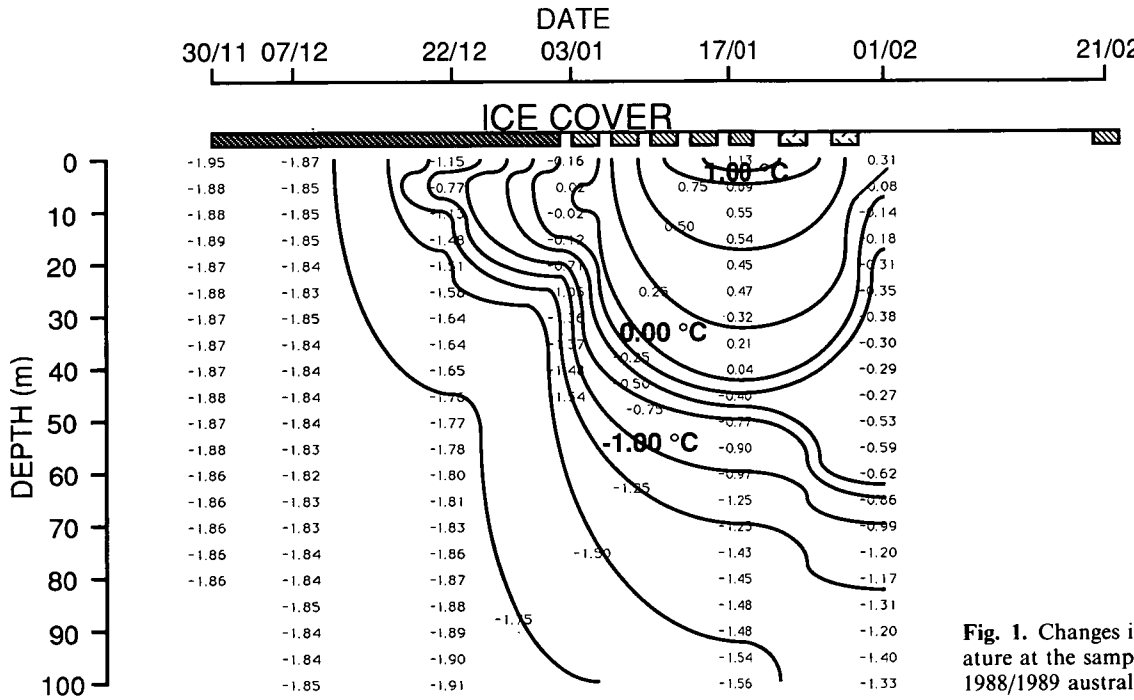


Fig. 1. Changes in ice cover and temperature at the sampling site during the 1988/1989 austral summer

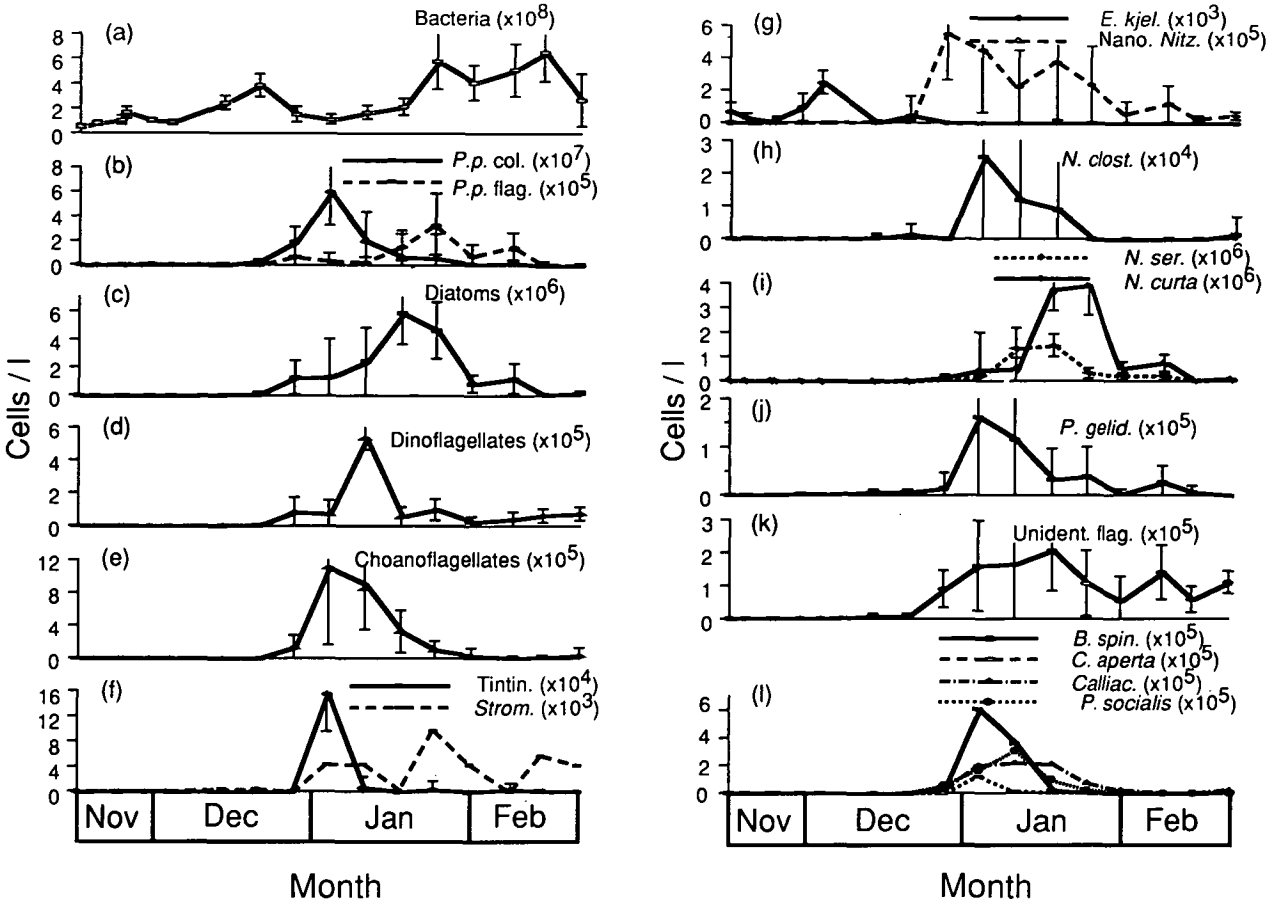


Fig. 2. changes in the concentration of **a** bacteria, **b** *Phaeocystis pouchetii* cells in the colonial (*P.p.* col.) and in the motile (*P.p.* flag.) phases of its life cycle, **c** total diatoms, **d** dinoflagellates, **e** total choanoflagellates, **f** tintinnids (Tintin.) and *Strombidium* spp. (*Strom.*), **g** *Entomoneis kjellmannii* (*E. kjel.*) and *nanoplanktonic Nitzschia* spp. (Nano. *Nitz.*), **h** *Nitzschia closterium* (*N. clost.*), **i** *N. seriata* (*N. ser.*) and *Nitzschia curta*, **j** *Pyramimonas gelidicola.* (*P. gelid.*), **k** unidentified flagellates (Unident. flag.) and **l** choanoflagellate species *Bicosta spinifera* (*B. spin.*), *Crinolina aperta* (*C. aperta*), *Calliacantha* spp. (*Calliac.*), *Parvicorbicula socialis* (*P. socialis*). Error bars indicate ± 1 standard deviation. Standard deviations for *Strombidium* were always greater than the mean and are not shown

Abundance of organisms

Bacterial abundance was essentially trimodal (Fig. 2a) showing a small peak in November which declined in early December. The concentration again rose until mid-December then declined rapidly until the end of the year whereupon it increased again to the highest values for the summer between 24/1/89 and 14/2/89 before again decreasing in late February.

Phaeocystis pouchetii occurs both as motile, scale-covered flagellates and in gelatinous colonies. The colonial and flagellate stages were observed in low numbers from the beginning of sampling and, although dominating phytoplankton numbers, remained in low abundance until mid December. The abundance of the colonial stage then increased dramatically to 6×10^7 cells $\cdot l^{-1}$ on 3/1/89 before decreasing at about the same rate (Fig. 2b) but still remained numerically dominant until 8/2/89. Though changes in concentration were not significant, motile cells of *P. pouchetii*, increase in number at the same time as the colonial form but declined until 10/1/89, during the bloom of the colonial stage, after which they increased to 3.4×10^5 cells $\cdot l^{-1}$ in late January (Fig. 2b).

Total diatom concentration also rose in mid December, but did not appear to increase between 27/12/88 and 3/1/89, the time of greatest colonial *Phaeocystis* abundance. Diatom concentration appeared to increase after 3/1/89, to a maximum of 5.9×10^6 cells $\cdot l^{-1}$ on 17/1/89 (Fig. 2c).

Taxonomic groups whose members consist partly or wholly of heterotrophs followed similar trends in abundance over the summer, with maxima in abundance during or immediately after the peak in *Phaeocystis* abundance. Dinoflagellate concentration began to increased in mid December, remained at approximately 7×10^4 cells $\cdot l^{-1}$ and persisted at around this concentration for the remainder of the summer with the exception of a further dramatic increase to a peak abundance of 5.4×10^5 cells $\cdot l^{-1}$ on 10/1/89 (Fig. 2d). Total choanoflagellates increased in number between 27/12/88 and 24/1/89,

reaching a maximum concentration of 1.2×10^6 cells $\cdot l^{-1}$ on 3/1/89 after which their abundance gradually declined (Fig. 2e).

The principal ciliates were tintinnids and *Strombidium* spp. (Fig. 2f). The tintinnid population was dominated by *Eutintinnus* spp. while *Codonellopsis* sp. was occasionally observed. *Eutintinnus* was not seen before 3/1/89 when its concentration rose to 1.6×10^5 cells $\cdot l^{-1}$. Its concentration then rapidly declined and beyond 10/1/89 remained less than 5×10^3 cells $\cdot l^{-1}$.

Strombidium spp. (mainly *S. sulcatus*) were the only other ciliates observed and differed from other heterotrophs in not peaking in abundance during or immediately after the *Phaeocystis* bloom (Fig. 2f). Their concentrations were never significantly above zero but low numbers were first observed on 3/12/90, increasing to approximately 5×10^3 cells $\cdot l^{-1}$ on the 3/1/90 and remained at around this level for the remainder of the sampling time with a peak on 24/1/89 of 10^4 cells $\cdot l^{-1}$.

Various species of diatoms proliferated at different times during the summer. Earliest was *Entomoneis kjellmanii*, a major component of the sea-ice community, which peaked on 3/12/88 but declined rapidly and was not seen for the rest of the summer (Fig. 2g). Nanoplanktonic *Nitzschia* spp. concentration, which was often comprised largely of *N. pseudonana*, reached their maximum on 27/12/89. This peak concentration of these cells coincided with declining sea-ice cover and may have been released from the sea-ice. Their concentration began a decline on 20/12/88 which continued throughout the remainder of the summer (Fig. 2g). *Nitzschia closterium* (Fig. 2h) was exceptional among the diatoms in that it reached maximum concentration between 28/12/88 and 17/1/89, during the bloom of *Phaeocystis*. The concentration of *N. curta* and morphologically similar chain-forming diatoms grouped within the species. *N. seriata* increased in concentration from 27/12/88 and attained maximum abundance on 17/1/89 (Fig. 2i), immediately followed by *Nitzschia curta*. *Nitzschia seriata* and *N. curta* were the major constituents of the diatom bloom. The minor constituents of the diatom assemblage, *Chaetoceros dichaeta*, *C. neglectum* and *C. simplex*, were most abundant during the *Phaeocystis* bloom. *Nitzschia cylindrus*, *Rhizosolenia* spp. and *Nitzschia frigida* contributed to the assemblage after the *Phaeocystis* peak.

Peak abundance of the prasinophyte *Pyramimonas gelidicola* (1.6×10^5 cells $\cdot l^{-1}$) on the 3/1/89, coinciding with the bloom of the colonial stage of *Phaeocystis*, and persisted longer (Fig. 2j). Auto- and heterotrophic flagellates, of which most were nanoplanktonic and the most numerous was *Micromonas* sp., increased in concentration from the 20/12/88, reaching a peak concentration of 2×10^5 cells $\cdot l^{-1}$ on 17/1/89 (Fig. 2k).

The choanoflagellate population was dominated by *Bicosta spinifera*, *Crinolina aperta*, *Calliacantha* spp. and *Parvicorbicula socialis*, the latter commonly in aggregates (Fig. 2l). The concentration of these species increased during the *Phaeocystis* bloom. *B. spinifera* and the previously unobserved *P. socialis* attained maximum concentrations on the 3/1/89, whereupon they declined rapidly. *C. aperta* and *Calliacantha* spp. persisted longer than *B. spinifera* and *P. socialis* and peaked in abundance on the 10/1/89.

Assemblages of organisms

Figures 3a and b show the samples that were grouped together from their species abundance using cluster analysis and MDS respectively. Separation of sample dates at a similarity of 0.87 in cluster analysis gave identical groupings to those obtained by MDS MDS results are adequately depicted in two dimensions (2-D) as stress, which measures the goodness of fit of the data to the model, only rose from 5.18 to 8.33 when plotted in 3-D and 2-D respectively. Group 1 contained samples between 15/11/88 and 5/12/88 when auto- and microheterotroph concentrations were low and bacterial concentrations moderate. Group 2 comprised two samples, 13/12/88 and 20/12/88. At these times phytoplankton concentrations were increasing towards the colonial *Phaeocystis* bloom, microheterotroph concentrations were low and bacterial concentration increasing. Group 3 contained samples from 27/12/88 to 14/2/89 and encompassed the bloom of phytoplankton and heterotrophs. The sole sample in group 4 was taken on the 21/2/89, the last sample of the summer when *P. pouchetii* was absent for the first time. *Pyramimonas gelidicola* and tintinnids were also absent and bacterial concentrations had declined from their peak.

Analysis of species groups using cluster analysis (Fig. 3c) and MDS (Fig. 3d) again gave six identical groups at a cluster similarity index of 0.69. Two dimensions was an adequate depiction of MDS as stress on the model only rose from 5.23 to 8.13 on going from 3-D to 2-D. Group 1 consisted of *E. kjellmanii* alone. This was the only species to attain maximum numbers in group 1 of the sample comparisons. Group 2 contained those species that had a relatively brief peak in abundance about the time of the *Phaeocystis* peak and which were present for most of the summer. These species were colonial *Phaeocystis*, *Nitzschia closterium*, *Pyramimonas gelidicola*, *Bicosta spinifera*, *Parvicorbicula socialis* and *Calliacantha* spp. Group 3 comprised partly or wholly autotrophic taxa namely, *Nitzschia seriata*, dinoflagelates, nanoplanktonic *Nitzschia* spp. and unidentified flagellates, the populations of which declined or increased only slowly during the *Phaeocysts* maximum. *Crinolina aperta* and tintinnids were very closely related to form group 4. Both species were essentially absent until their peak on the 3/1/89 followed by a rapid decline. Group 5, containing flagellate *Phaeocystis* and *Nitzschia curta*, showed the same decline in growth rates as group 3 but their peak in abundance occurred on 24/1/89, three weeks after the *Phaeocystis* peak. Species group 6, which was composed of *Strombidium* and bacteria, perisisted from early season until the end of sampling with peak abundance in January and February.

Organic carbon and nitrogen

Three main peaks of DOC occurred during the summer (Fig. 4a). The first on 24/11/88 occurred before the appear-

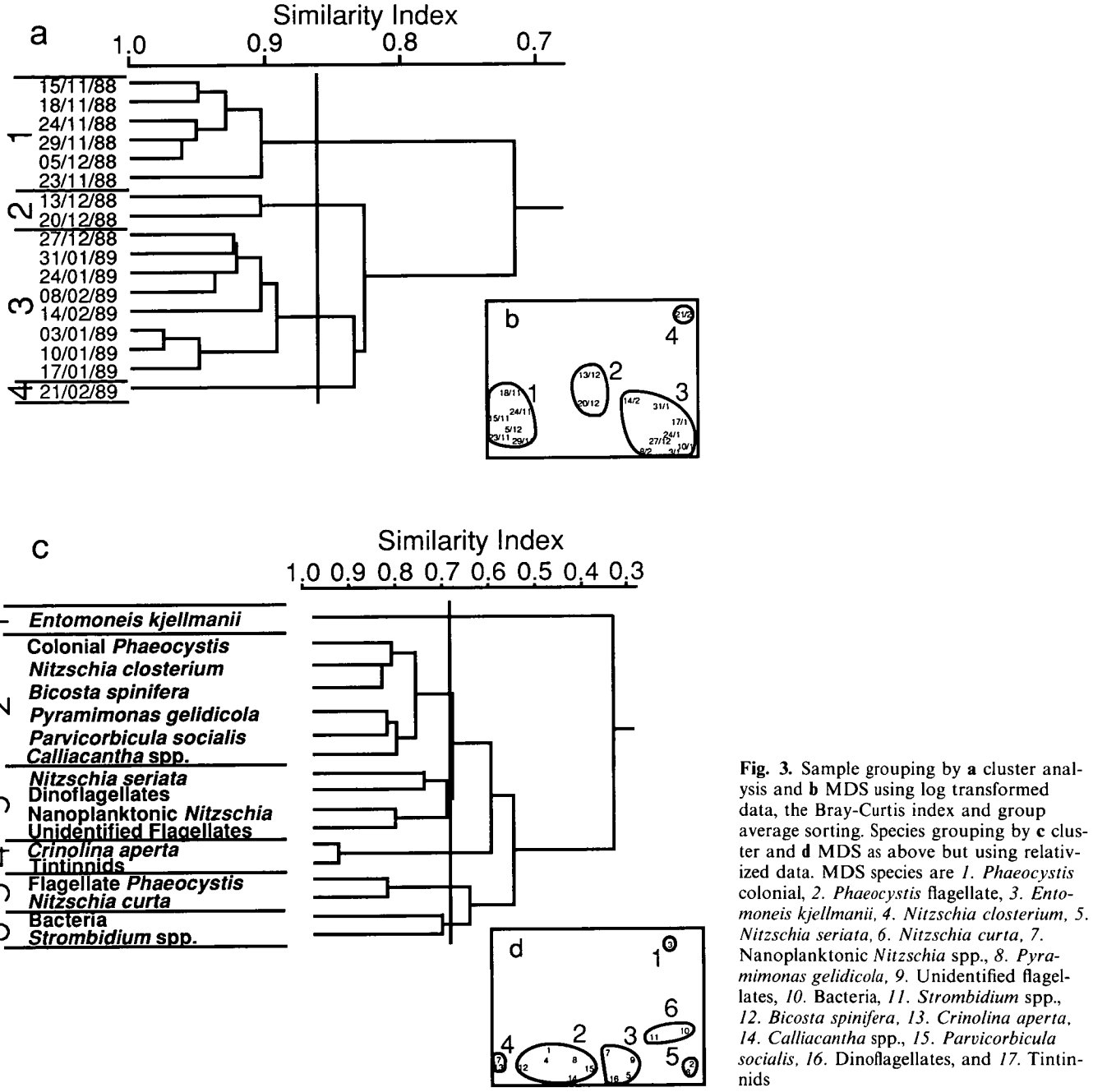


Fig. 3. Sample grouping by **a** cluster analysis and **b** MDS using log transformed data, the Bray-Curtis index and group average sorting. Species grouping by **c** cluster and **d** MDS as above but using relativized data. MDS species are *1. Phaeocystis* colonial, *2. Phaeocystis* flagellate, *3. Entomoneis kjellmanii, 4. Nitzschia closterium, 5. Nitzschia seriata, 6. Nitzschia curta, 7.* Nanoplanktonic *Nitzschia* spp., *8. Pyramimonas gelidicola, 9.* Unidentified flagellates, *10.* Bacteria, *11. Strombidium* spp., *12. Bicosta spinifera, 13. Crinolina aperta, 14. Calliacantha* spp., *15. Parvicorbicula socialis, 16.* Dinoflagellates, and *17.* Tintinnids

ance of any major primary producers in the summer and is likely to have been released from the sea-ice. This DOC peak coincided also with peaks of POC (Fig. 4b) and PCHO (Fig. 4c). The major peak of DOC, which reached a maximum in excess of 100 $mg \cdot l^{-1}$, POC and PCHO coincided with the bloom of colonial *Phaeocystis* and persisted from around 20/12/88 to 31/1/89. The third peak of DOC (80 $mg \cdot l^{-1}$) occurred on 14/2/89, a time of declining POC and PCHO. The concentration of POC and PON declined steadily from the major peak of 760 $\mu g \cdot l^{-1}$ and 107 $\mu g \cdot l^{-1}$ respectively on 10/1/89 (Fig. 4b). Before 20/12/88 concentrations of POC and PON were too low to calculate the C:N ratio. After this the molar C:N ratio was approximately 7:1, approximately that of the Redfield ratio, and remained at this value until 8/2/89 after which it increased to around 13:1 (Fig. 4b).

Discussion

The timing of peaks in abundance of bacteria, autotrophic and heterotrophic protists and changes in the concentration of DOC, POC and PON appear closely related during summer at this coastal Antarctic site. The concentrations of taxa previously reported from this site suggest

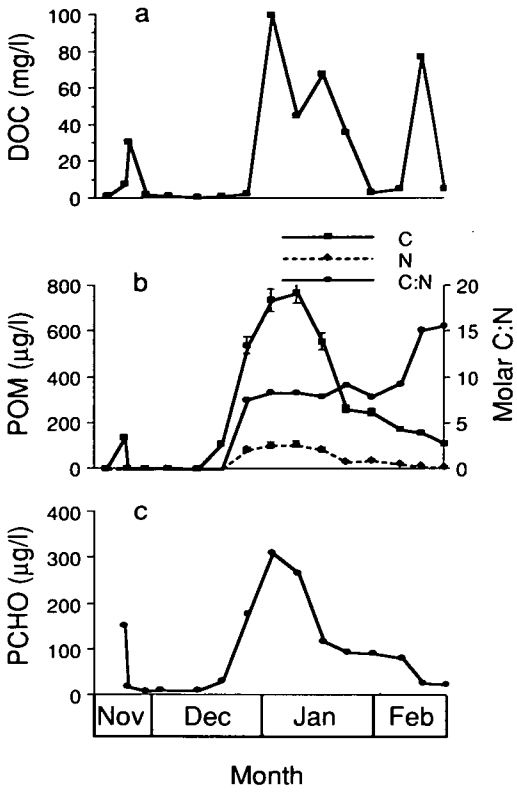


Fig. 4. Changes in the concentration of **a** dissolved organic carbon (*DOC*), **b** particulate organic matter (*POM*) showing carbon (*C*), nitrogen (*N*) and the molar ratio of carbon to nitrogen (*C:N*) and **c** the concentration of glucose equivalent particulate carbohydrate (*PCHO*). The absolute concentration of *DOC* on 3/1/89 is unknown but was greater than 100 mg·l^{-1}

an interannual recurrence of similar species and abundance (Perrin et al. 1987; Gibson et al. 1990; Marchant and Perrin 1990). Thus the biotic and abiotic events determining the community at this site appear to be spatially and temporally coupled and it appears that this study, based on a single site and depth, is likely to be representative of recurring protistan community dynamics in the coastal area off Davis Station. Concentrations changed rapidly on occasions during this study. This clearly does not infer rates of production from repeated sampling of a discrete population but instead indicates associations and interactions of organisms in water masses as they pass the fixed sampling site and provides information on the successional sequences as planktonic succession is, by necessity, defined in these terms (eg. Jeffrey 1981).

Bacterioplankton

The November peaks of bacteria, *Entomoneis kjellmanii* DOC, POC and PCHO, when the sea was entirely ice covered and the water column was thermally unstructured, were probably due to release of material from the sea-ice (Garrison and Buck 1989b). *Phaeocystis* secretes much of its photoassimilated carbon (Guillard and Hellebust 1971) and these extracellular products are a suitable substrate for bacteria (Eberlein et al. 1985; Veldhuis et al. 1986; Davidson and Marchant 1987; Verity et al. 1988). However, the bloom of this alga after 20/12/88 coincides with a decline in bacterial concentration confirming observations by Laanbroek et al. (1985) and Lancelot and Billen (1984) that bacterial concentrations are depressed by actively growing *Phaeocystis*. In contrast, Gibson et al. (1990) found that the December minimum in bacterial numbers was not concurrent with the peak of this alga and was attributed to grazing alone. The decline in bacterial abundance between 20/12/88 and 17/1/89 occurred at the time of PCHO abundance and the seasonal maximum of DOC. This was probably initiated by the release of a bacteriocide by actively growing *Phaeocystis* (Sieburth 1960; Davidson and Marchant 1987) and reinforced by microflagellate grazing. The abundance of DOC (in excess of 100 mg·l^{-1}) during the *Phaeocystis* bloom demonstrates the incapacity of bacteria to fully utilize this substrate.

In late January the bacterial concentration increased greatly following the decline in microheterotroph concentrations. This agrees with Fenchel (1982); Garrison and Buck (1989a) and Sherr et al. (1989) who have shown microheterotroph grazing is a principal determinant of bacterial mortality. A peak in DOC concentration in early February may have resulted from the decline of the coastal phytoplankton bloom. This DOC peak coincided with peak bacterial numbers, suggesting that a direct relationship between the two may be sustainable when microheterotroph grazing is minimal. However, it remains possible that the preservation and counting techniques used in this study failed to detect other nanoplanktonic bacterial grazers.

The delay between the substrate production by phytoplankton and the bacterioplankton peak at a single sample site was found to be about 10 days in the North Sea (Billen and Fontigny 1987), 11 to 18 days off Newfoundland (Pomeroy and Deibel 1986) and about one month in Antarctic waters (Billen et al. 1987). The discrepancy in correlation of phyto- and bacterioplankton biomass showed that, in comparison with temperate waters, bacterial response to substrate availability in polar and subpolar waters was delayed. Our data also showed a one month delay between the peak concentrations of phytoplankton and bacteria but clearly indicate that the relationship is not simple.

Protists

Protist abundance attained its maximum as sea ice cover declined. Garrison and Buck (1989b) observed inoculation of the water column with protists from the ice biota. Such a release could have contributed significantly to the sudden increase in concentration of *Phaeocystis*, organic carbon and protozoa that we observed during the ice breakup in early January. The slight increase in concentration of flagellate *Phaeocystis* to around 8×10^4 cells·l^{-1} early in the season followed by a decline during the bloom of the colonial stage support observations by Kornmann (1955)

and Kayser (1970) that the flagellate stage may be a source of colonies. In addition, as reported by Verity et al. (1988), the flagellate stage was also observed being liberated from colonies at the end of the colonial bloom. This life-cycle change led to the increase in the concentration of the flagellate stage in late January.

The peculiar physiology of *Phaeocystis* strongly influences the structure and function of communities in which it predominates (Lancelot et al. 1987). Our data support the observation of Jones and Haq (1963); Smayda (1973); Chang (1983); Barnard et al. (1984); Admiraal and Venekamp (1986); Bätje and Michaelis (1986); Veldhuis et al. (1986) and Weisse et al. (1986) that *Phaeocystis* has an antagonistic effect on other phytoplankton species. Those autotrophs whose numbers remained constant or declined during the *Phaeocystis* bloom occurred separately in both cluster analysis and MDS (Fig. 3c and d). Production of UV-B absorbing pigments by Antarctic colonial *Phaeocystis* (Marchant et al. 1991) may have profound effects on the timing of its abundance in these waters. In addition, manganese concentrations in Southern Ocean are very low (Martin et al. 1990) and the high manganese accumulation reported for colonial *Phaeocystis* (Morris 1971; Davidson and Marchant 1987; Lubbers et al. 1990) may influence the succession of Antarctic marine autotrophs.

At the time of greatest protist abundance (cluster and MDS sample group 3) species were divided into groups 4, 2 and 3 representing those species that peaked during the *Phaeocystis* maximum only, high *P. pouchetii* and post-*P. pouchetii* bloom environment respectively. While the abundance of most autotrophic populations did not increase during the *Phaeocystis* bloom, protozoa proliferated. The coincidence of *Phaeocystis* and tintinnids has also been observed by Admiraal and Venekamp (1986) who reported blooms of tintinnids immediately following *P. pouchetii* blooms. They demonstrated tintinnids grazing on *Phaeocystis* and concluded that microfaunal grazing may limit the duration of the *Phaeocystis* bloom. Dinoflagellates, of which a large proportion may be heterotrophic (Garrison and Buck 1989b), peaked at this time. It is unknown whether they grazed *Phaeocystis*. The peak in choanoflagellates that coincided with the *Phaeocystis* bloom attained concentrations approximately an order of magnitude higher than those reported for the Weddell Sea by Buck and Garrison (1988). Choanoflagellates would be relying on bacterial grazing as the major source of nutrition. However, bacterial production rates would be required to ascertain this as bacterial concentration was low at the time and may have been inhibited by the bacteriocidal products of *Phaeocystis*. Direct uptake by microzooplankton of POC and DOC produced by autotrophs has been reported (Fenchel 1987; Sherr 1988; Marchant 1990). It remains possible that the microzooplankton maximum at the time of highest autotrophic POC and DOC substrate availability and low bacterial numbers indicates direct uptake of these substrates as a source of nutrition.

After the *Phaeocystis* peak the concentrations of microzooplankton, namely ciliates, heterotrophic dinoflagellates and choanoflagellates, declined sharply. This was not due to insufficient carbon as the DOC and POC concentrations in these samples was only slightly lower than those in early January and bacterial concentrations had increased. Hewes et al. (1985) suggested that microzooplankton form an important link to higher trophic levels in Antarctic waters and what Lampert (1978) described as "sloppy feeding" by zooplankton may have contributed to the increase in DOC at the time of low microzooplankton abundance. Our data indicate that the environment created by a *Phaeocystis* bloom may increase the emphasis on microzooplankton as the link to higher trophic levels.

The ciliate *Strombidium* is the exception to the pattern observed in the other microzooplanktonic groups. MDS and cluster analysis demonstrate that this organism is poorly linked with the abundance of other protists. As a grazer, its concentration may be responding to the abundance of small diatoms and flagellates. However, together with its capacity to function as a facultative mixotroph (Stoecker et al. 1987), the range of particle size available to it probably make it less dependent on the trophic structures applied to the other microheterotrophs.

Organic compounds

Discrimination of POC and DOC produced by *Phaeocystis* is equivocal. Most of the biovolume of its blooms is due to the colony matrix of which significant proportions can be lost by filtration (Verity and Smayda 1989; Veldhuis and Admiraal 1985; Lancelot 1984; Bölter and Dawson 1982). Filtration to dryness and rinsing with ammonium carbonate employed in this study would probably have lost much of the mucilage in the particulate fraction, hence significantly underestimating the POC. This is borne out by the relatively low PCHO concentration given that the DOC indicates very high carbon exudation rates. Though filtration pressures were low (< 100 mm Hg) some mucilage is likely to have been lost to the DOC fraction. The sample for DOC analysis was taken at the beginning of the filtration when contamination by mucilage would be minimal. The extremely rapid changes in organic carbon and nitrogen (requiring production rates around $20\,g\,C \cdot m^{-3} \cdot day^{-1}$) are so high that this production could not have occurred in situ and serve to illustrate the transport of communities to the sample site.

Measured POC and PON concentrations obtained during this study are similar to the $822\,\mu g \cdot l^{-1}$ and $87.6\,\mu g \cdot l^{-1}$ respectively obtained during a bloom of 2.8×10^7 cells $\cdot l^{-1}$ along the North Wales coast (Claustre et al. 1990). DOC concentrations of up to $20\,mg \cdot l^{-1}$ were measured in an Antarctic *Phaeocystis* bloom by Bölter and Dawson (1982) and our cell concentrations are greater than any previously reported. POC concentrations were over two orders of magnitude less than the DOC at the peak of the *Phaeocystis* bloom and this persisted, though to a lesser extent, for the remainder of the period when *Phaeocystis* dominated. It is evident from the POC, PCHO and DOC concentrations we observed that organic substrates, particularly DOC, are available far in excess of their utilization. *Phaeocystis* is renowned for release of large quantities of DOC. In the North Sea and

English Channel the DOC released upon breakup of its blooms forms extensive deposits of foam on northern European beaches (Eberlein et al. 1985).

Claustre et al. (1990) found that the greatest part of the biomass of *Phaeocystis* blooms was lost from the food web linking phytoplankton to copepods. Wassmann et al. (1990) concluded that grazing on *Phaeocystis* was insufficient to control the magnitude of its bloom. In Antarctica Bölter and Dawson (1982) also found that heterotrophy was insufficient to utilize the DOC pool during the periods of intense primary production of a *Phaeocystis* bloom. Our data showed that more than 99% of the organic carbon existed as DOC and that this was largely unutilized at the sample site. Thus, previously published models of carbon flux which include *Phaeocystis* blooms (Billen and Fontigny 1987; Billen et al. 1987) and those describing flux within individual colonies (Lancelot and Mathot 1985) differ greatly from this study. From our results, DOC appears to comprise a far greater proportion of the *Phaeocystis* production than reported in the northern hemisphere.

Conclusions

Phaeocystis apparently influences the abundance of other autotrophic species. The massive concentrations of carbon present during a bloom of *P. pouchetii* are apparently not significantly exploited by bacteria as their numbers are low at this time. The bloom of choanoflagellates, dinoflagellates and tintinnids during and immediately following the *Phaeocystis* maximum suggests that a large proportion of the carbon passing to higher trophic levels is channelled through the microheterotrophs. Heterotrophy is advantageous to Antarctic organisms surviving some nine months of the year under low light levels. When light and carbon are at their maximum it appears possible that these organisms may utilise this resource, largely unconstrained by bacterial activity. As a dominant member of the phytoplankton, a major source of carbon and a bacteriocidal source *Phaeocystis pouchetii* is also a crucial determinant of the heterotrophic community. This study emphasises the importance of this organism in Antarctic coastal waters.

Acknowledgements. We thank D. Eslake for sample collection, J. Dalpont, CSIRO Division of Oceanography for DOC analyses, G. Hosie for advice on statistics and D. Garrison and J. Parslow for their comments on the manuscript. We acknowledge financial support from the Australian Research Council.

References

Admiraal W, Venekamp LAH (1986) Significance of tintinnid grazing during blooms of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. Neth J Sea Res 20:61–66

Bätje M, Michaelis H (1986) *Phaeocystis pouchetii* blooms in the East Frisian coastal waters (German Bight, North Sea). Mar Biol 93:21–27

Barnard WR, Andreae MO, Iverson RL (1984) Dimethylsulfide and *Phaeocystis pouchetii* in the south Bering Sea. Cont Shelf Res 3:103–113

Billen G, Fontigny A (1987) Dynamics of a *Phaeocystis* -dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. Mar Ecol Prog Ser 37:249–257

Billen G, Lancelot C, Mathot S (1987) Ecophysiology of phyto- and bacterioplankton growth in the Prydz Bay area during the austral summer 1987. II. Bacterioplankton activity. In: Proc Belg Nat Colloq Antarct Res. Prime Ministers Services-Service Policy Office, Brussels, pp 133–146

Bölter M Dawson R (1982) Heterotrophic utilisation of biochemical compounds in Antarctic waters. Neth J Sea Res 16:315–332

Buck KR, Garrison DL (1983) Protists from the ice-edge region of the Weddell Sea. Deep-Sea Res 30:1261–1277

Buck KR, Garrison DL (1988) Distribution and abundance of choanoflagellates (Acanthoecidae) across the ice edge zone in the Weddell Sea, Antarctica. Mar Biol 98:263–269

Chang FH (1983) The mucilage producing *Phaeocystis pouchetii* (Prymnesiophyceae) cultured from the 1981 "Tasman Bay Slime". NZ J Mar Freshw Res 17:165–168

Claustre H, Poulet SA, Williams R, Marty J-C, Coombs S, Ben Mlih F, Hapette AM, Martin-Jezequel V (1990) A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. J Mar Biol Assoc UK 70:197–207

Davidson AT, Marchant HJ (1987) Binding of manganese by Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release. Mar Biol 95:481–487

Eberlein K Leal MT Hammer KD Hickel W (1985) Dissolved organic substances during a *Phaeocystis pouchetii* bloom in the German Bight (North Sea). Mar Biol 89:311–316

Fenchel T (1982) Ecology of heterotropic microflagellates. IV. Quantitative occurrence and importance as bacterial consumers. Mar Ecol Prog Ser 9:35–42

Fenchel T (1987) Ecology of protozoa. Science Tech Publishers, Madison Springer Berlin Heidelberg New York Tokyo

Field JG, Clarke KR, Warwick RM (1982) A practical strategy for analysing multispecies distribution patterns. Mar Ecol Prog Ser 8:37–52

Fryxell GA Kendrick GA (1988) Austral spring microalgae across the Weddell Sea ice edge: spatial relationships found along a northward transect during AMERIEZ 83. Deep-Sea Res 35:1–20

Fuhrman JA, Azam F (1980) Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl Environ Microbiol 39:1085–1095

Garrison DL, Buck KR (1989a) Protozooplankton in the Weddell Sea, Antarctica: abundance and distribution in the ice-edge zone. Polar Biol 9:341–351

Garrison DL, Buck KR (1989b) The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsula regions. Polar Biol 10:211–219

Garrison DL, Buck KR, Fryxell GA (1987) Algal assemblages in the antarctic pack ice and in ice-edge plankton. J Phycol 23:564–572

Gibson JAE, Garrick RC, Burton HR (1990) The annual cycle of bacterial numbers at an inshore site near the antarctic continent. Proc NIPR Symp Polar Biol 3:16–22

Guillard RRL, Hellebust JA (1971) Growth and the production of extracellular substances by two strains of *Phaeocystis pouchetii*. J Phycol 7:330–338

Hanson RB, Lowery HK, Shafer D, Sorocco R, Pope DH (1983a) Microbes in Antarctic water of the Drake Passage: Vertical patterns of substrate uptake, productivity and biomass in January 1980. Polar Biol 2:179–188

Hanson RB, Shafer D, Ryan T, Pope DH, Lowery HK (1983b) Bacterioplankton in Antarctic Ocean waters during late austral winter: abundance, frequency of dividing cells, and estimates of production. Appl Environ Microbiol 45:1622–1632

Hewes CD, Holm-Hansen O, Sakshaug E (1985) Alternate carbon pathways at lower trophic levels in the antarctic food web. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient

cycles and food webs (Proc 4th SCAR Symp Antarct Biol). Springer, Berlin, pp 277–283
Jeffrey SW (1981) Phytoplankton ecology-with particular reference to the Australian region. In: Clayton MN King RJ (eds) Marine botany: an Australian perspective. Longman Cheshire, Melbourne, pp 241–292
Jones PGW, Haq SM (1963) The distribution of *Phaeocystis* in the eastern Irish Sea. J Cons 28:8–20
Kayser A (1970) Experimental-ecological investigations on *Phaeocystis pouchetii* (Haptophyceae): cultivation and waste water test. Helgol Wiss Meeresunters 20:195–212
Kogure K, Fukami K, Simidu U, Taga N (1986) Abundance and production of bacterioplankton in the Antarctic. Mem Natl Inst Polar Res, Spec Issue 40:414–422
Kornmann VP (1955) Beobachtungen an *Phaeocystis* -Kulturen. Helgol Wiss Meeresunters 5:218–233
Kottmeier ST, Sullivan CW (1990) Bacterial biomass and production in pack ice of antarctic marginal ice edge zones. Deep-Sea Res 37:1311–1330
Laanbroek HJ Verplanke JC, Visscher PRM de, Vuyst R de (1985) Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, the Netherlands. Mar Ecol Prog Ser 25:1–11
Lampert W (1978) Release of DOC by grazing zooplankton. Limnol Oceanogr 23: 831–834
Lancelot C (1984) Metabolic changes in *Phaeocystis pouchetii* (Hariot) Lagerheim during the spring bloom in Belgian coastal waters. Estuar Coast Shelf Sci 18:593–600
Lancelot C, Billen G (1984) Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. Limnol Oceanogr 29:721–730
Lancelot C, Mathot S (1985) Biochemical fractionation of primary production by phytoplankton in Belgian coastal waters during short- and long-term incubations with ^{14}C-bicarbonate. II. *Phaeocystis pouchetii* colonial population. Mar Biol 86:227–232
Lancelot C, Billen G, Sournia A, Weisse T, Colijn F, Veldhuis MJW, Davies A, Wassmann P (1987) *Phaeocystis* blooms and nutrient enrichment in the continental coastal zones of the North Sea. Ambio 16:38–46
Lipski M (1987) Variations of physical conditions, nutrients and chlorophyll *a* contents in Admiralty Bay (King George Island, South Shetland Islands, 1979). Polar Res 8:307–332
Lubbers GW, Gieskes, WWC, Castilho P del, Salomons W, Bril J (1990) Manganese accumulation in the high pH microenvironment of *Phaeocystis sp.* (Haptophyceae) colonies from the North Sea. Mar Ecol Prog Ser 59:285–293
Marchant HJ (1990) Grazing rate and particle size selection by the choanoflagellate *Diaphanoeca grandis* from the sea-ice of Lagoon Saroma-ko, Hokkaido. Proc NIPR Symp Polar Biol 3:1–7
Marchant HJ, Perrin RA (1990) Seasonal variation in abundance and species composition of choanoflagellates (Acanthoecideae) at antarctic coastal sites. Polar Biol 10:499–505
Marchant HJ, Davidson AT, Kelly GJ (1991) UV-B protecting pigments in the marine alga *Phaeocystis pouchetii* from Antarctica. Mar Biol 109:391–395
Marshall SM, Orr AP (1962) Carbohydrate as a measurement of phytoplankton. J Mar Biol Assoc UK 42:511–519
Martin JH Gordon RM, Fitzwater SE (1990) Iron in Antarctic waters. Nature 345:156–158
Morris AW (1971) Trace metal variations in sea water of the Menai Straits caused by a bloom of *Phaeocystis*. Nature 233:427–428
Perrin R, Lu P, Marchant HJ (1987) Seasonal variation in marine phytoplankton and ice algae at a shallow Antarctic coastal site. Hydrobiologia 146:33–46
Pomeroy LR, Deibel D (1986) Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science 233:359–361
Porter KG, Feig YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
Satoh H, Fukami K, Watanabe K, Takahashi E (1989). Seasonal changes in heterotrophic bacteria under fast ice near Syowa station, Antarctica. Can J Microbiol 35:329–333
Sherr GB (1988) Direct use of high molecular weight polysaccharide by heterotrophic flagellates. Nature 335:348–351
Sherr BF, Sherr EB, Pedros-Alio C (1989) Simultaneous measurement of bacterioplankton production and protozoan bactivory in estuarine water. Mar Ecol Prog Ser 54:209–219
Sieburth JMcN (1960) Acrylic acid, an "antibiotic" principal in *Phaeocystis* blooms in Antarctic waters. Science 132:676–677
Smayda T (1973) The growth of *Skeletonema costatum* during a winter-spring bloom in Narragansett Bay, RI. Norw J Bot 20:219–247
Stoecker DK, Michaels AE, Davis LH (1987) Large proportion of marine planktonic ciliates found to contain functional chloroplasts. Nature 326:790–792
Veldhuis MJW, Admiraal W (1985) Transfer of photosynthetic products in gelatinous colonies of *Phaeocystis pouchetii* (Haptophyceae) and its effect on the measurement of excretion rate. Mar Ecol Prog Ser 26:301–304
Veldhuis MJW, Colijn F, Venekamp LAH (1986) The spring bloom of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. Neth J Sea Res 20:37–48
Verity PG, Smayda TJ (1989) Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytolankton for *Acartia* spp. (Copepoda): ingestion, egg production and growth of nauplii. Mar Biol 100:161–171
Verity PG, Villareal TA, Smayda TJ (1988) Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. II. The role of life-cycle phenomena in bloom termination. J Plankton Res 10:749–766
Wassmann P, Vernet M, Mitchell BG, Rey F (1990) Mass sedimentation of *Phaeocystis pouchetii* in the Bering Sea. Mar Ecol Prog Ser 66:183–195
Weisse T, Grimm N, Hickel W, Martens P (1986) Dynamics of *Phaeocystis pouchetii* blooms in the Wadden Sea of Sylt (German Bight, North Sea). Estuar Coast Shelf Sci 23:171–182

Reprinted from

Proceedings of the NIPR Symposium on
Polar Biology, No. 7

COMPARATIVE IMPACT OF *IN SITU* UV EXPOSURE ON PRODUCTIVITY, GROWTH AND SURVIVAL OF ANTARCTIC *PHAEOCYSTIS* AND DIATOMS

Andrew T. DAVIDSON and Harvey J. MARCHANT

National Institute of Polar Research, Tokyo, January 1994

Proc. NIPR Symp. Polar Biol., 7, 53–69, 1994

COMPARATIVE IMPACT OF *IN SITU* UV EXPOSURE ON PRODUCTIVITY, GROWTH AND SURVIVAL OF ANTARCTIC *PHAEOCYSTIS* AND DIATOMS

Andrew T. DAVIDSON and Harvey J. MARCHANT

Australian Antarctic Division, Channel Highway, Kingston, Tasmania, 7050, Australia

Abstract: Depletion of stratospheric ozone over Antarctica enhances UV-B (280–320 nm) radiation reaching the Earth's surface during spring. UV-B influences the growth and survival of marine phytoplankton. The near surface UV irradiance, *in situ* growth and primary production of the prymnesiophyte *Phaeocystis* c.f. *pouchetii* (HARIOT) LAGERHEIM and three diatoms were measured during UV exposure. Survival, growth and cell diameter were also determined after exposure. The flagellate stage in the life cycle of *Phaeocystis* was the only organism examined that suffered mortality as a result of natural UV exposure, however, UV-A (320–400 nm) was responsible for most of this mortality. Interspecific differences in production, cell concentration and growth were observed at sublethal irradiances. Such differences may lead to changes in phytoplankton species composition.

1. Introduction

Ozone depletion over Antarctica has occurred between September and November since the mid-1970s (STOLARSKI *et al.*, 1986). This depletion has increased UV-B irradiances (280–320 nm) reaching the Earth's surface during spring to levels at least as high as those at the summer solstice (FREDERICK and SNELL, 1988; LUBIN *et al.*, 1989). Sea-ice algae contribute 10–50% of the primary production in some areas (VOYTEK, 1989) and phytoplankton inhabiting shallow mixed depths of the marginal ice zone (MIZ) support 25–67% of the phytoplanktonic production in the Southern Ocean (SMITH and NELSON, 1986). The Antarctic sea ice in spring can be sufficiently transparent to UV that biologically significant doses are received by the ice algal community (TRODAHL and BUCKLEY, 1989). The mixed depth during blooms in the MIZ can be 10 m or less for up to 6 days (VETH, 1991). UV-B penetrates to depths in excess 50 m in Antarctic waters (GIESKES and KRAAY, 1990; KARENTZ and LUTZE, 1990; SMITH *et al.*, 1992). Thus, much production by phytoplankton in the Southern Ocean occurs in environments vulnerable to UV-B radiation at a time when irradiances at these wavelengths are enhanced by stratospheric ozone depletion.

Phytoplankton form the base of the Antarctic food web and sustain the wealth of life for which the Southern Ocean is renown (AINLEY *et al.*, 1986). Exposure of phytoplankton to UV-B radiation reduces photosynthesis, growth, survival, nutrient uptake and photosynthetic pigment concentrations, effects

motility and phototactic orientation and increases mutagenesis in DNA and proteins (*e.g.* JITTS *et al.*, 1976; LORENZEN, 1979; CALKINS and THORDARDOTTIR, 1980; WORREST *et al.*, 1981; WORREST, 1983; DÖLER, 1984, 1985, 1987; HÄDER, 1986, 1987, 1988; JOKIEL and YORK, 1984; KARENTZ *et al.*, 1991; MARCHANT *et al.*, 1991). This has lead to concern about the effect of elevated UV-B levels on the Antarctic ecosystem. Opinions regarding the magnitude of the effect range from insignificant (HOLM-HANSEN *et al.*, 1989) to catastrophic (EL-SAYED *et al.*, 1990).

Interspecific variation in survival, growth and repair responses to UV-B exposure is reportedly high (CALKINS and THORDARDOTTIR, 1980; WORREST *et al.*, 1981; JOKIEL and YORK, 1984; KARENTZ *et al.*, 1991; SMITH *et al.*, 1992), even within a single genus (MITCHELL and KARENTZ, 1990). This has lead to the proposal that increased UV-B irradiance is likely to alter the species composition of phytoplankton communities in favor of those species with greater tolerance (WORREST *et al.*, 1981; WORREST 1983; JOKIEL and YORK, 1984; EL-SAYED *et al.*, 1990; KARENTZ, 1990, 1991; HÄDER and WORREST, 1991; KARENTZ *et al.*, 1991; MARCHANT and DAVIDSON, 1991; HELBLING *et al.*, 1992). Long term exposure of natural phytoplankton assemblages to *in situ* UV irradiances reportedly changes the community composition (WORREST *et al.*, 1981; BOTHWELL *et al.*, 1993). This could effect the trophic interactions and carbon flux rates of Antarctic waters (EL-SAYED *et al.*, 1990; KARENTZ, 1990; HÄDER and WORREST, 1991; KARENTZ *et al.*, 1991; MARCHANT and DAVIDSON, 1991) and may have far reaching effects on the Southern Ocean ecosystem (EL-SAYED *et al.*, 1990). However, there is little direct evidence that increased UV-B irradiance as a result of stratospheric ozone depletion has caused changes in phytoplankton species composition of the Southern Ocean.

CALKINS and THORDARDOTTIR (1980) suggested that temperate and sub-polar diatoms possess little reserve capacity to cope with increased UV-B exposure. THOMSON *et al.* (1980) and HANNAN *et al.* (1980) showed that UV-B could significantly reduce the growth rate of marine diatoms and in Antarctic waters. EL-SAYED *et al.* (1990) concluded that Antarctic phytoplankton are currently UV stressed and are likely to be seriously affected by any increase in UV radiation. In contrast, studies of North American phytoplankton by GALA and GIESY (1991) and HOBSON and HARTLEY (1983) found little inhibition of production by UV-B and DAVIDSON *et al.* (1994) found that selected species of Antarctic diatoms, though variable in their response, sustained no significant mortality until UV-B exposures were increased to levels almost an order of magnitude greater than those currently experienced in Antarctic surface waters. While the prospects for diatoms under increasing UV-B irradiances are uncertain, tolerance of nanoplankton to UV-B exposure is little known but apparently low (EL-SAYED *et al.*, 1990; KARENTZ *et al.*, 1991).

UV-A wavelengths are not enhanced by ozone depletion, however, they have been found to be a major factor in depressing rates of photosynthesis and growth (*e.g.* JITTS *et al.*, 1976; JOKIEL and YORK, 1984; MASKE, 1984; BUHLMANN *et al.*, 1987; HELBLING *et al.*, 1992). HOLM-HANSEN *et al.* (1989) found that in near surface Antarctic waters approximately 50% of inhibition of photosynthesis

was due to UV-A. The greater penetration of the water column by UV-A than UV-B meant that UV-A was responsible for most of the photoinhibition in these waters (HOLM-HANSEN, 1990). Long term exposures of phytoplankton have also shown UV-A is responsible for almost all inhibition of phytoplankton growth (JOKIEL and YORK, 1984).

The nanoplanktonic prymnesiophyte *Phaeocystis* c.f. *pouchetii* is arguably the most abundant and widespread phytoplankter of the Antarctic marine ecosystem (FRYXELL and KENDRICK, 1988). It is a frequent member of the ice-algal assemblage and one of the first species to bloom in the top few meters of the water column (GARRISON *et al.*, 1987; FRYXELL and KENDRICK, 1988). Together with diatoms, principally of the genus *Nitzschia, Phaeocystis* frequently dominates the phytoplankton of the ice-edge bloom and plays a pivotal role in determining the structure and function of the planktonic community (GARRISON *et al.*, 1987; FRYXELL and KENDRICK, 1988; GARRISON and BUCK, 1989; DAVIDSON and MARCHANT, 1992a). Any UV mediated change in the abundance of *Phaeocystis* relative to diatoms would significantly alter the particle size, form and availability of carbon to higher trophic levels and is likely to change vertical carbon flux rates (MARCHANT and DAVIDSON, 1991). Here we report the *in situ* primary production, growth and survival of Antarctic isolates of *Phaeocystis* and selected species of diatoms and their post-irradiance growth at an Antarctic coastal site.

2. Materials and Methods

Unialgal cultures of *Chaetoceros simplex* OSTENFELD, *Stellarima microtrias* (EHRENBERG) HASLE and SIMS, *Nitzschia curta* (V.H.) HASLE and a *Phaeocystis* c.f. *pouchetii* (HARIOT) LAGERHEIM were isolated from Prydz Bay, Antarctica in 1991/92 and were maintained in culture under cool white fluorescent light at photosynthetically active radiation (PAR) intensity of 5.11 Wm^{-2}. *C. simplex, S. microtrias* and *N. curta* were grown in f/2 medium (GUILLARD and RYTHER, 1962) and a mixed flagellate and colonial life stage culture of *Phaeocystis pouchetii* was grown in GP5 (LOEBLICH and SMITH, 1968). An exponentially growth phase culture of each species of was diluted 1:6 with fresh nutrient medium two days before *in situ* incubation. Immediately before irradiation the cultures were thoroughly mixed and two hundred and fifty m*l* of each species transferred to each of three Whirlpak bags which transmitted light above 220 nm (PAR, UV-A and UV-B treatment). One bag remained unscreened while the remainder were screened with mylar (which transmitted wavelengths above 320 nm–PAR and UV-A treatment) or polycarbonate (which transmitted wavelengths above 370 nm–PAR treatment). Like PRÉZELIN and SMITH (1993) we found no evidence of inhibition of growth or photosynthesis by UV-B induced toxicity of Whirlpaks (HOLM-HANSEN and HELBLING, 1993). Interspecific differences in growth and photosynthesis were species specific rather than treatment dependant. Bags were then incubated at 0.30 m depth in near-shore waters off Davis between 19th February and 26th February 1992.

A further seven 50 m*l* subsamples of each species were transferred to 100 m*l*

Whirlpak bags for primary production incubations. Three bags were screened as above, one was screened with opaque black plastic as a dark bag control and a further three were immediately acidified with 200 μl of 6 N HCl as time zero blanks. Primary production was estimated using the methods of SCHINDLER *et al.* (1972) modified after GRIFFITH (pers. comm.). At the conclusion of the production incubation a 7 m*l* subsample from each Whirlpak was transferred to a 20 m*l* scintillation vial and acidified as above. The vials were then shaken at 200 rpm for 2 hours to remove inorganic ^{14}C. Counts were performed in Lumagel using a LKB 1215 Rackbeta II liquid scintillation counter. Estimates of count efficiency were performed each sample day before performing decay counts. The mean of triplicate time zero blanks and dark bag uptake were subtracted from counts in calculation of primary production. *In situ* incubations were performed at 0.30 m depth for 4 hours between 10.30 and 12.30 solar time. Determination of primary production by each species and under each light treatment was repeated after 4 and 8 days *in situ* incubation. The light treatment of each primary production incubation was the same as that from which the subsample was removed.

Surface UV-A and UV-B irradiance was integrated *in situ* using an International Light IL 1700 Radiometer. Primary calibration of detector response was made using a National Institute of Standards and Technology intercomparison package (NIST Test # 534/240436-88) with further calibration using four International Light primary transfer standards.

A 5 m*l* subsample of each *in situ* incubated treatment for each species was inoculated into 30 m*l* of fresh growth medium. These cultures were returned to culture maintenance conditions for estimation of growth rate and survival and will henceforth be referred to as "ongrowth" cultures. A further 10 m*l* was removed at each sample time and fixed with Lugol's iodine for estimation of cell concentration using inverted microscope cell counts over 15 replicate fields. Cell concentration in ongrown cultures was estimated 3 and 9 days after subculturing and the growth rate of the control culture then used to calculate the number of surviving cells immediately after irradiation from the final cell concentration in irradiated treatments (DAVIDSON *et al.*, 1994). Calculations ensure that only viable cells capable of contributing to population growth are included in the survival of each species under each light treatment. After 2, 4, and 8 days *in situ* exposure subsamples were removed from each 250 m*l* Whirlpak and the *in situ* cell concentration, survival and rate of ongrowth again estimated.

The equivalent spherical diameters of *P. pouchetii* flagellate and colonial cells were measured microscopically using a Zeiss Photomicroscope II at 1000× magnification. A total of 200 equivalent spherical diameters were measured from each light treatment which had been irradiated for 8 days and ongrown for a further 9 days.

3. Results

Surface UV-A and UV-B irradiances were integrated during the duration of the 8 days *in situ* incubation (Figs. 1, 3 and 4) and during each 4 hour primary

production incubation (Figs. 5 and 6). Surface irradiances were high during the first 2 days of incubation as were irradiances during the primary production incubations. Between days 2 and 4 conditions were frequently overcast and surface irradiances were low, particularly at UV-B wavelengths. Irradiances during primary production incubation were similarly low. Between day 4 and 8 integrated UV-A and UV-B irradiance increased again and surface irradiances integrated over the duration of the primary production incubation were the highest observed.

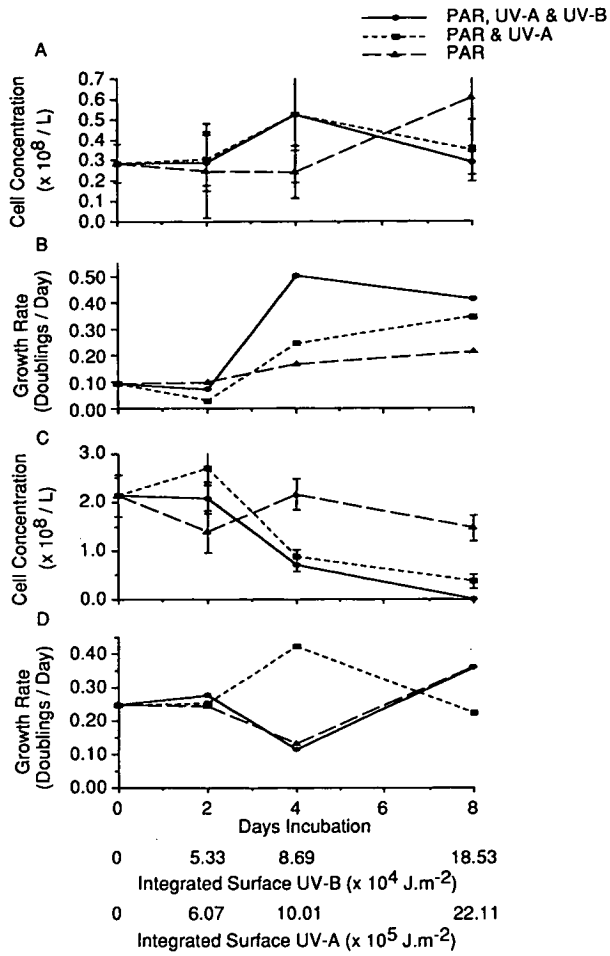


Fig. 1. Colonial Phaeocystis *(A) cell concentration during* in situ *irradiation and (B) growth rate of cells incubated for 0, 2, 4 and 8 days, subcultured, returned to culture maintenance conditions and ongrown for 9 days. Flagellate stage* Phaeocystis *(C) cell concentration during* in situ *irradiation and (D) growth rate after irradiation (as above). Growth rate calculated after* VERITY *et al. (1988). Total integrated UV-A and UV-B dose at each* in situ *sample period are given. Error bars represent standard deviation.*

The concentration of colonial *Phaeocystis* changed little during *in situ* incubations (Fig. 1A). Samples which received UV-B in the irradiance did not differ significantly from those that received PAR and UV-A. Only in the incubation which received PAR alone may cell concentration have increased but this never differed significantly from UV exposed treatments. Exposure of colonial *Phaeocystis* to unscreened solar irradiance (PAR, UV-A and UV-B) for periods of more than 2 days greatly increased their rate of post-irradiance ongrowth (Fig. 1B). Colonial cells which received PAR and UV-A also showed a marked but lesser promotion of growth rate while growth of PAR irradiated control samples showed little increase in growth rate with incubation time.

The concentration of flagellate cells fell as a result of *in situ* UV radiation.

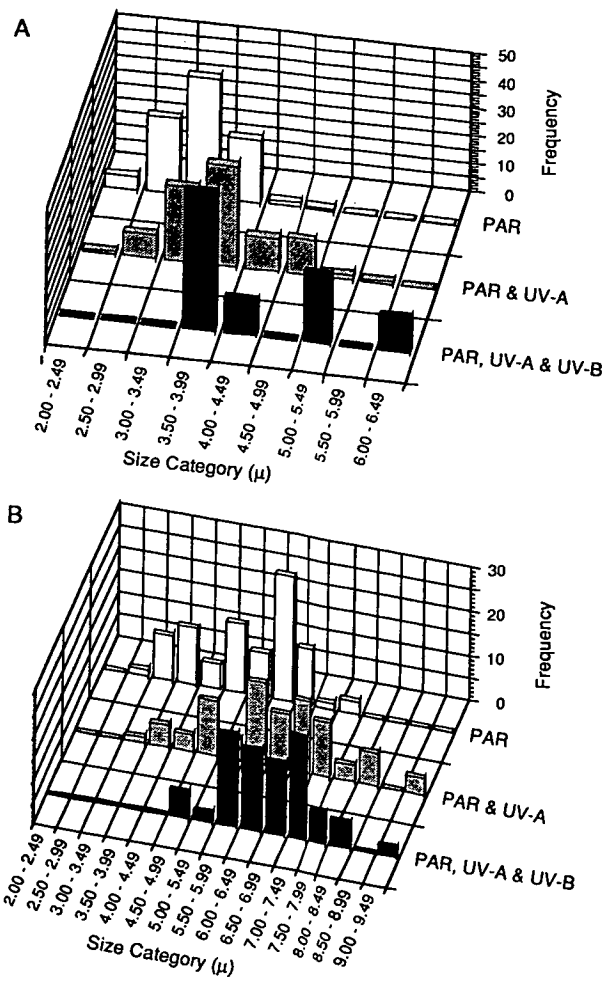


Fig. 2. Cell size distribution of (A) flagellate and (B) colonial cells incubated in situ *at 0.30 m depth for 8 days exposed to PAR, PAR and UV-A or PAR, UV-A and UV-B subcultured and ongrown in culture maintenance conditions for 9 days.*

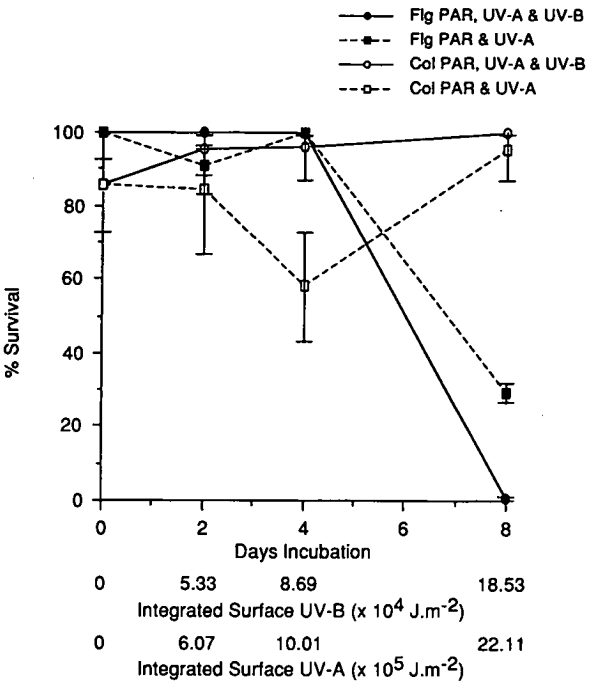


Fig. 3. Percent survival of unscreened (Flg PAR, UV-A & UV-B) or mylar screened (Flg PAR & UV-A) flagellate stage and unscreened (Col PAR, UV-A & UV-B) or mylar screened (Col PAR & UV-A) colonial stage Antarctic Phaeocystis *culture during near surface* in situ *incubations. Error bars represent standard error calculated after* Zar *(1984).*

Flagellate concentrations in the PAR irradiated treatment remained approximately constant (Fig. 1C). Cells subject to PAR and UV-A declined to around 20% of their original numbers over the 8 day period while flagellate concentrations exposed to PAR, UV-A and UV-B declined at a similar rate but where almost absent after 8 days incubation. The rate of ongrowth of the flagellate stage after irradiation changed little with time irrespective of irradiance treatment (Fig. 1D). The only exception was the PAR and UV-A treatment after 4 days incubation, the reasons for which are uncertain.

The cell diameter of the colonial and flagellate cells increased with addition of UV-A and UV-B to the irradiance (Fig. 2A and B). Mean flagellate cell diameter in cultures receiving PAR were 3.18 μm (Fig. 2A). This increased to 3.71 μm with addition of UV-A to the exposure and reached 4.50 μm when also exposed to UV-B. The mean cell diameter of the colonial stage was 5.03 μm after exposure to PAR only (Fig. 2B). This increased to 6.18 μm with the introduction of UV-A and further increased to 6.59 μm after exposure to UV-A and UV-B.

Exposure of colonial stage *Phaeocystis* to natural irradiances over a period of 8 days caused no decline in survival (Fig. 3). Survival of flagellate stage

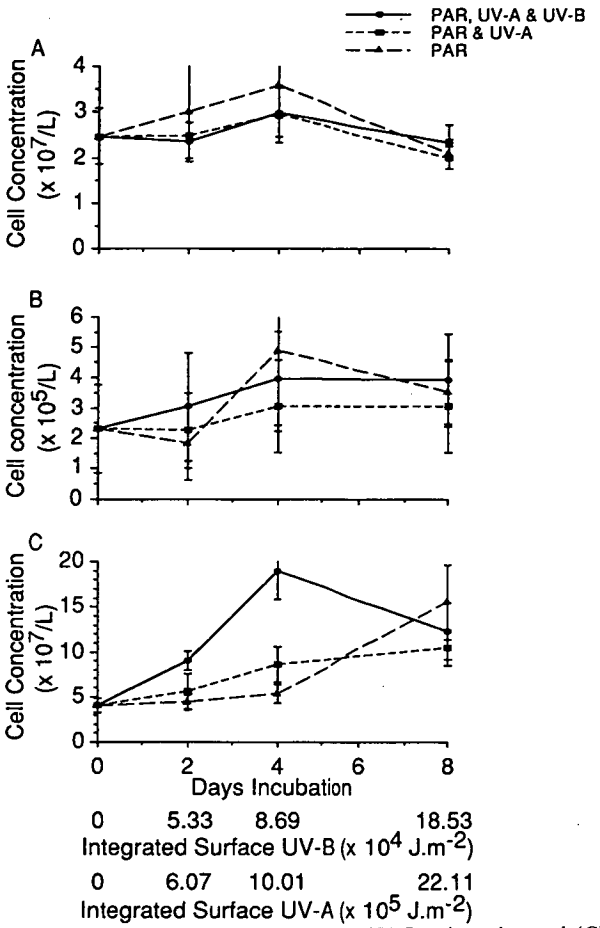


Fig. 4. Cell concentration of (A) C. simplex, *(B)* S. microtrias *and (C)* N. curta *exposed to PAR, PAR and UV-A or PAR, UV-A & UV-B during near surface* in situ *incubations. Total integrated UV-A and UV-B dose at each* in situ *sample period are given. Error bars represent standard deviation*

Table 1. Percent survival of PAR and UV-A or PAR,UV-A and UV-B irradiated diatoms exposed to near surface in situ *irradiance for 8 days calculated after* DAVIDSON et al. *(1994). L1 and L2 represent upper and lowe standard errors calculated after* ZAR *(1984).*

Species	PAR & UV-A			PAR, UV-A & UV-B		
	Mean	L1	L2	Mean	L1	L2
S. microtrias	95.88	99.87	86.77	93.53	98.55	85.28
C. simplex	99.17	99.97	96.04	98.59	99.91	95.70
N. curta	100.00	100.00	100.00	100.00	100.00	100.00

Table 2. Growth rate of diatoms after 8 days near surface in situ *exposure to PAR, PAR and UV-A or PAR, UV-A and UV-B, subcultured and ongrown in culture maintenance conditions for 9 days. Growth rate calculated after* VERITY et al. *(1988).*

Species	Growth rate		
	PAR	PAR & UV-A	PAR, UV-A & UV-B
S. microtrias	0.263	0.218	0.122
C. simplex	0.674	0.336	0.266
N. curta	0.253	0.289	0.657

Phaeocystis also remained high for the first 4 days incubation but declined markedly between days 4 and 8. The decline was greatest when cultures were exposed to the PAR, UV-A and UV-B but a major decline was also observed in the treatment with PAR and UV-A.

The concentration of *C. simplex* and *S. microtrias* cells did not increase significantly during *in situ* incubation (Fig. 4A and B). Concentrations of *N. curta* did significantly increase in all treatments. The greatest increase was observed in the unscreened treatment during the first 4 days of irradiation after which the concentration declined toward day 8 (Fig. 4C). None of the diatom species exhibited any significant decline in the survival as a result of UV irradiance (Table 1). Interspecific differences were observed in the growth rate of cultures established and ongrown after irradiance treatments (Table 2). Growth of *S. microtrias* and *C. simplex* declined with the addition of UV-A and UV-B to the irradiance. UV-B was responsible for the greatest decline in the growth rate of *S. microtrias* while the greatest decline in growth rate of *C. simplex* was caused by UV-A. *N. curta* showed a promotion of growth rate in the unscreened treatment similar to that observed for *Phaeocystis*. Unlike *Phaeocystis*, little promotion of growth rate resulted from addition of UV-A to the irradiance.

Total photosynthetic rates of *Phaeocystis* only declined slightly with incubation time and little difference was observed between the irradiance treatments (Fig. 5A). The carbon fixation rate per cell in the PAR screened treatment also exhibited little change with time (Fig. 5B), however, fixation rates per cell in treatments which receiving UV-A or UV-A and UV-B increased rapidly. This resulted from the decrease in flagellate cell concentration (Fig. 1C). In addition, the irradiance treatment and the flux rate during the production incubation appear to have little effect on the rate of production by the colonial stage (Fig. 5A). The diatom species investigated showed differing responses in production to the irradiance treatment. Although rates of production were frequently lowest in treatments which received UV-B, inhibition of photosynthesis was only slight. The rate of production per cell by the diatom species investigated was not reflected in changes in cell concentration during *in situ* incubation. Little difference was observed in primary production per cell of *C. simplex* between light treatments, however, the production by each cell approximately doubled during

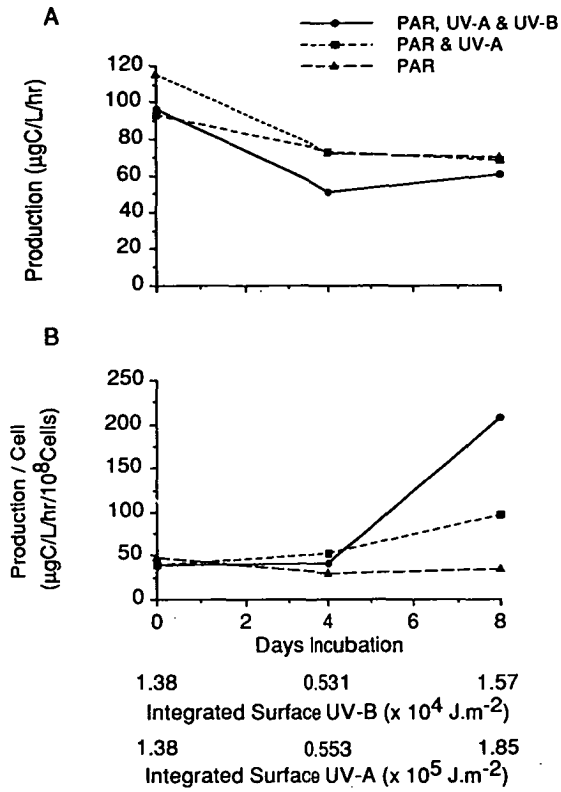


Fig. 5. The rate of (A) primary production and (B) production per cell by cultured Phaeocystis *taken from near surface* in situ *incubations. 50 ml sub-samples were removed from polycarbonate, mylar or unscreened light treatments and replaced* in situ *beneath the same screen for 4 hr incubations to estimate primary production. Surface UV-A and UV-B irradiance was integrated for the duration of the production incubations.*

in situ incubation (Fig. 6A). Production per cell by *S. microtrias* appeared to decline slightly during incubation (Fig. 6B), while that by *N. curta* declined by approximately 90% in all treatments (Fig. 6C).

4. Discussion

4.1. Survival

Flagellate stage *Phaeocystis* was the only organism examined which demonstrated a significant decline in cell concentration during *in situ* exposure and survival after irradiation. UV-A was responsible for most of this decline. JOKIEL and YORK (1984) found that long term inhibition of growth was due almost entirely to UV-A. Our results indicate that it can also account for most of the mortality. Addition of UV-B to the irradiance further reduced the cell concentration of the flagellate stage but differences were slight and only significant after 8

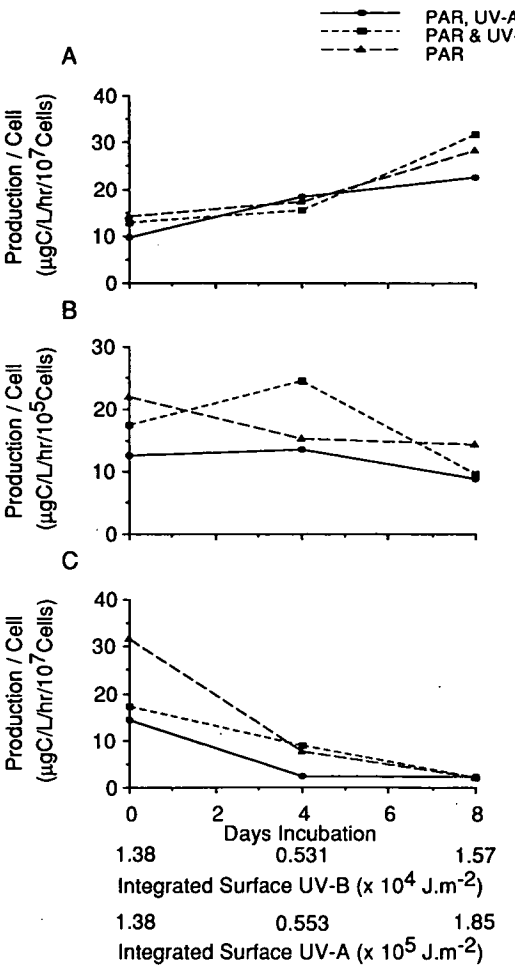


Fig. 6. The rate of *primary production per cell by (A)* C. simplex, *(B)* S. microtrias *and (C)* N. curta *during near surface* in situ *incubations performed as for Phaeocystis.*

days irradiation. KARENTZ *et al.* (1991) and CALKINS and THORDARDOTTIR (1980), indicate that UV-B induced mortality would act as a selective pressure on the species composition of the phytoplankton community. As UV-A irradiances are not significantly enhanced as a result of ozone depletion, our results indicate that no major decline in *Phaeocystis* or diatom abundance as a result of UV-B induced mortality is likely.

Antarctic colonial *Phaeocystis* possesses high concentrations of UV absorbing compounds which provided substantial protection from UV radiation (MARCHANT *et al.*, 1991). The flagellate stage lacks these compounds and exhibited greater vulnerability to UV radiation. Our results support this finding. Antarctic near surface UV irradiances are sufficient to cause mortality in flagellate *Phaeocystis* populations but the colonial stage maintains its photosynthetic production during

exposure to UV and exhibits increased growth and cell size and high survival after irradiation with UV-A and UV-B.

4.2. Growth

UV is reportedly responsible for significant decreases in the growth rate of phytoplankton (THOMSON *et al.*, 1980; HANNAN *et al.*, 1980; JOKIEL and YORK, 1984; DÖHLER, 1984, 1985). WORREST *et al.* (1981) attributed this to interspecific differences in genetic limits of photoadaptation. During *in situ* incubations using diluted exponentially growth phase cultures we found only *N. curta* sustained significant growth. This suggests that growth may have been inhibited as a result of PAR, however, JOKIEL and YORK (1984) found high levels of PAR were not inhibitory to growth. Alternatively, features of the *in situ* environment such as temperature may have reduced growth rates. SMITH *et al.* (1992) found that growth of *Phaeocystis* (presumably colonial) in Antarctic waters was inhibited by inclusion of UV-B in the natural solar irradiance while the growth of *Chaetoceros socialis* was not. We did not observe significant inhibition of colonial *Phaeocystis* growth by UV-B but this may have been due to our use of monospecific nutrient enriched cultures or differences in experimental methods and strain.

In situ exposure of *Phaeocystis* to UV resulted in an increase in cell diameter in both the flagellate and colonial life stages of subcultures established immediately after irradiation and allowed to grow in culture maintenance conditions for a further 9 days. An increase in cell size may be caused by inhibition of cell division (BADOUR, 1968) or an increase in light intensity (THOMSON *et al.*, 1991). The concentration of flagellate cells decreased in irradiances treatments including UV-A or UV-A and B but their growth rate after irradiation did not differ markedly between irradiance treatments indicating that flagellate cells which survived irradiation were able to sustain normal growth. No significant change in colonial cell concentration was observed in any of the irradiance treatments during *in situ* irradiation and those that received UV-A or UV-A and UV-B exhibited enhanced growth rates after irradiation. Thus, unlike BADOUR (1968), the observed increase in cell size as a result of exposure to UV radiation appear not to be as a result of inhibition of cell division. The increase in cell size observed by THOMSON *et al.* (1991) were reversible after 12 hours while the increase we observed was wavelength dependant and persisted for at least the 9 days of ongrowth. Our results indicate a sustained change in cell metabolism of UV irradiated *Phaeocystis*. Changes in size of the flagellate cells may, however, also reflect UV induced changes in flagellate cell stage (KORNMANN, 1955; L. PEPERZAK pers. comm.) or formation of flagellates from the colonial stage (VELDHUIS *et al.*, 1986; VERITY *et al.*, 1988; DAVIDSON and MARCHANT, 1992b).

Exposure of *N. curta* to PAR, UV-A and UV-B caused a rapid increase in cell concentrations for the first 4 days of incubation suggesting high UV tolerance by this species. UV irradiances between days 4 and 8 were high. During this time the concentration of *N. curta* in this treatment declined suggesting that exposure of the cells beyond an upper threshold becomes inhibitory to their growth or that the UV exposure may impose cumulative stress on cell physiology which is

expressed only after extended periods of irradiation (CALKINS and THORDARDOTTIR, 1980; JOKIEL and YORK, 1984; DÖHLER, 1984; VOSJAN *et al.*, 1990; MARCHANT *et al.*, 1991).

Interspecific differences were observed in the growth rate of cultures established and ongrown after irradiance treatments. Ongrowth of *N. curta* showed the division rate of the PAR, UV-A and UV-B irradiated treatment for this species was more than twice that of other treatments despite its rates of primary production during incubation being low. To sustain growth after irradiation the photosynthetic rate of *N. curta* must recover rapidly, however, the differences in the rate of ongrowth by *N. curta* must largely reflect UV-B induced effects on processes other than photosynthesis. DAVIDSON *et al.* (1994) suggests the possibility of UV-B being involved in repair of UV-A related damage. This may explain the higher growth rate of the unscreened treatment than that receiving UV-A. The reason for the lower growth rate of the PAR irradiated ongrowth culture is unclear but, like colonial *Phaeocystis*, exposure to UV may promote growth after irradiation. Laboratory studies will be undertaken to further investigate the apparently contradictory responses of *N. curta* to UV-B exposure. Ongrowth of *S. microtrias* showed greatest reduction in growth rate as a result of UV-B irradiance. That of *C. simplex* was reduced most by UV-A but declined further with addition of UV-B to the irradiance. The reduced rate of ongrowth by these species may, at least in part, reflect the degree of inhibition of photosynthesis by UV during *in situ* incubation.

The photobiological strategy favored as a result of UV-B exposure would depend on the duration and intensity of the irradiance received. Though the diatoms we have examined survive high UV irradiances for a short time (DAVIDSON *et al.*, 1994) their long term survival and growth during and after irradiation may not advantage them over species that appear more vulnerable. For example, *S. microtrias* is able to survive UV-B intensities approximately an order of magnitude higher than that of *Phaeocystis* (DAVIDSON *et al.*, 1994), however, it grows little better than *Phaeocystis* during *in situ* incubation and irradiation with UV-B results in depression of growth after exposure. The rate of ongrowth for *Phaeocystis* after exposure to UV-B irradiation was approximately 3 times that of the PAR irradiated culture and this species would likely be favored at sublethal irradiances.

4.3. Production

UV is widely reported as being inhibitory to photosynthesis (*e.g.* LORENZEN, 1979; JITTS *et al.*, 1976; JOKIEL and YORK, 1984; WORREST *et al.*, 1981; SMITH and BAKER, 1989; WORREST, 1986; HÄDER and WORREST, 1991; VOYTEK, 1989). Estimates of inhibition by near surface UV-B irradiances range from 15–30% while UV-A resulted in a further decline of around 50% (HELBLING *et al.*, 1992; HOLM-HANSEN, 1990; HOLM-HANSEN *et al.*, 1989; MASKE, 1984). In Antarctic waters the increase in UV-B as a result of ozone depletion apparently result in a reduction of at least 6–12 % in primary production (SMITH *et al.*, 1992). We found inhibition of production was variable, probably as a result of variations in

tolerance and photoadaptive ability of each species and changes in the *in situ* irradiance received. Photoinhibition was frequently greatest for treatments which received UV-B in the irradiance but differences between light treatments were slight and percent inhibition seldom reached the magnitude reported above. However, we used nutrient enriched monospecific cultures in our investigation and the lower sensitivity may reflect the high nutrient environment (CULLEN and LESSER, 1991)

The colonial *Phaeocystis* cell concentration remained relatively constant during the 8 days of irradiation. Exposure of flagellate cells to UV for periods exceeding 2 days significantly reduced their concentration in culture. However, in comparison with the PAR irradiated control, the rate of production in UV irradiated treatments did not markedly decline and the production per *Phaeocystis* cell greatly increased. Although no size fractionated production was conducted to separate the flagellate and colonial stages of *Phaeocystis*, the colonial stage in the life cycle of this alga appears largely responsibly for photosynthesis during *in situ* incubation. This may be as a result of possessing UV absorbing compounds (MARCHANT *et al.*, 1991) which protect the photosynthetic apparatus and/or the sustained changes in physiology as a result of UV exposure. *C. simplex* was the only diatom which increased its rate of photosynthesis per cell during *in situ* incubation suggesting photoadaptation of this species to the near surface light environment. Primary production by *N. curta* declined markedly but this was apparently largely due to PAR irradiance rather than UV wavelengths. This contrasts with the finding of previous authors that PAR has little inhibitory effect upon photosynthesis (JOKIEL and YORK, 1984; BUHLMANN *et al.*, 1987).

5. Conclusion

The net effect of survival, photo-protective mechanisms, photosynthetic rate and growth would determine the niche available to each species in the UV environment. The nature and duration of UV exposure in Antarctic waters is yet to be fully determined. The shallow blooms of the MIZ, which are responsible for much of the primary production in the Southern Ocean, appear vulnerable to increased UV-B radiation as a result of stratospheric ozone depletion (MARCHANT and DAVIDSON, 1991). Interspecific differences in the responses of the phytoplankton to UV exposure have led to the suggestion that species or strains possessing greater tolerance to UV will be favored (HÄDER and WORREST, 1991; KARENTZ, 1991; MARCHANT and DAVIDSON, 1991). However, our results indicate that the interaction of UV intensity, dose and the photobiology of each species is complex and the impact on the organisms is not great. The consequent changes in phytoplankton species composition may be sufficiently slow or slight that they are undiscernible from spatial and interannual variability.

Acknowledgments

We gratefully acknowledge John GIBSON and Andrew MCMINN for their comments on the manuscript and Jeff HUNT, Paul SYNNOT, Lionel WHITEHORN, Peter SPRUNK and Fiona SCOTT for their assistance with field operations.

References

AINLEY, D. G., FRASER, W. R., SULLIVAN, C. W., TORRES, J. J., HOPKINS, T. L. and SMITH, W. O. (1986): Antarctic mesopelagic micronekton: Evidence from seabirds that pack ice affects community structure. Science, **232**, 847–849.

BADOUR, S. S. (1968): Experimental separation of cell division and silica shell formation in *Cyclotella cryptica*. Microbiology, **62**, 17–33.

BOTHWELL, M. L., SHERBOT, D., ROBERGE, A. C. and DALEY, R. J. (1993): Influence of natural ultraviolet radiation on lotic periphytic diatom community growth, biomass accrual, and species composition: short-term versus long-term effects. J. Phycol., **29**, 24-35.

BUHLMANN, B., BOSSARD, P. and UEHLINGER, U. (1987): The influence of longwave ultraviolet radiation (UV-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton. J. Plankton Res., **9**, 935–943.

CALKINS, J. and THORDARDOTTIR, T. (1980): The ecological significance of solar UV radiation on aquatic organisms. Nature, **283**, 563–566.

CULLEN, J. J. and Lesser, M. P. (1991): Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: results for a marine diatom. Mar. Biol., **111**, 183-190.

DAVIDSON, A. T. and MARCHANT, H. J. (1992a): Protist abundance and carbon concentration during a *Phaeocystis*-dominated bloom at an Antarctic coastal site. Polar Biol., **12**, 387–395.

DAVIDSON, A. T. and MARCHANT, H. J. (1992b): The biology and ecology of *Phaeocystis* (Prymnesiophyceae). Progress in Phycological Research, Vol. 8, ed. by F. E. ROUND and D. J. CHAPMAN. Bristol, Biopress, 1–45.

DAVIDSON, A. T., BRAMICH, D., MARCHANT, H. J. and MCMINN, A. (1994): Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms. to be published in Mar. Biol.

DÖHLER, G. (1984): Effect of UV-B radiation on the marine diatoms Lauderia annulata and *Thalassiosira rotula* grown in different salinities. Mar. Biol., **83**, 247–253.

DÖHLER, G. (1985): Effect of UV-B radiation (290-320 nm) on the nitrogen metabolism of several diatoms. J. Plant Physiol., **118**, 391–400.

DÖHLER, G. (1987): Effect of irradiation on nitrogen metabolism in marine diatoms and phytoplankton. Oceanis, **13**, 487–493.

EL-SAYED, S. Z., STEPHENS, F. C., BIDIGARE, R. R. and ONDRUSEK, M. E. (1990): Effect of ultraviolet radiation on Antarctic marine phytoplankton. Antarctic Ecosystems; Ecological Change and Conservation, ed. by K. R. KERRY and G. HEMPEL. Berlin, Springer, 379–385.

FREDERICK, J. E. and SNELL, H. E. (1988): Ultraviolet radiation levels during the antarctic spring. Science, **241**, 438–440.

FRYXELL, G. A. and KENDRICK, G. A. (1988): Austral spring microalgae across the Weddell Sea ice edge: Spatial relationships found along a northward transect during AMERIEZ 83. Deep Sea Res., **35**, 1–20.

GALA, W. R. and GIESY, J. P. (1991): Effects of ultraviolet radiation on the primary production of natural phytoplankton assemblages in Lake Michigan. Ecotoxicol. Environ. Saf., **22**, 345-361.

GARRISON, D. L. and BUCK, K. R. (1989): The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsular regions. Polar Biol., **10**, 211-219.

GARRISON, D. L., BUCK, K. R. and FRYXELL, G. A. (1987): Algal assemblages in the antarctic pack ice and in ice-edge plankton. J. Phycol., **23**, 564–572.

GIESKES, W. W. C. and KRAAY, G. W. (1990): Transmission of ultraviolet light in the Weddell Sea:

Report of the first measurements made in the Antarctic. BIOMASS Newsl., **12**, 12–14.

GUILLARD, R. R. L. and RYTHER, J. H. (1962): Studies of the marine plankton diatoms *Cyclotella nana* HUSTEDT and *Detonula confervaceae* (CLEVE) GRAN. Can. J. Microbiol., **8**, 229–239.

HÄDER, D.-P. (1986): Effects of solar and artificial UV radiation on motility and phototaxis in the flagellate *Euglena gracilis*. Photochem. Photobiol., **44**, 651–656.

HÄDER, D.-P. (1987): Effects of UV-B radiation on photomovement in the desmid, *Cosmarium cucumis*. Photochem. Photobiol., **46**, 121–126.

HÄDER D.-P. (1988): Ecological consequences of photomovement in microorganisms. Photochem. Photobiol., **1** (B), 385–414.

HÄDER, D.-P. and WORREST, R. C. (1991): Effects of enhanced solar ultraviolet radiation on aquatic ecosystems. Photochem. Photobiol., **53**, 717–725.

HANNAN, P. J., SWINNERTON, J. W., LAMONTAGNE, R. A. and PATOUILLET, C. (1980): Effect of UV-B on algal growth rate and trace gas production. Aquatic Toxicology, ed. by J. G. EATON *et al.* American Society for Testing and Materials, 177–190 (ISBN 0-686-76097-2).

HELBLING, E. W., VILLAFANE, V., FERRARIO, M. and HOLM-HANSEN, O. (1992): Impact of natural ultraviolet radiation on specific marine phytoplankton species. Mar. Ecol. Prog. Ser., **80**, 89–100.

HOBSON, L. A. and HARTLEY, F. A. (1983): Ultraviolet irradiance and primary production in a Vancouver Island fjord, British Columbia, Canada. J. Plankton Res., **5**, 325–331.

HOLM-HANSEN, O. (1990): Effects of ultraviolet-B and ultraviolet-A on photosynthetic rates on Antarctic phytoplankton. Antarct. J. U. S., **25**, 177–178.

HOLM-HANSEN, O. and HELBLING, E. W. (1993): Polythene bags and solar ultraviolet radiation. Science, **259**, 534.

HOLM-HANSEN, O., MITCHELL, B. G. and VERNET, M. (1989): Ultraviolet radiation in antarctic waters: Effects on rates of primary production. Antarct. J. U. S., **24**, 177–178.

JITTS, H. R., MOREL, A. and SAIJO, Y. (1976): The relation of oceanic primary production to available photosynthetic irradiance. Aust. J. Mar. Freshwater Res., **27**, 441–454.

JOKIEL, P. L. and YORK R. H., Jr. (1984): Importance of ultraviolet radiation in photoinhibition of microalgal growth. Limnol. Oceanogr., **29**, 192–199.

KARENTZ, D. (1990): Ecological considerations of the Antarctic ozone hole in the marine environment. Effects of solar ultraviolet radiation on biogeochemical dynamics in aquatic environments, ed. by N. V. BLOUGH and R. G. ZEPP. 137–140

KARENTZ, D. (1991): Ecological considerations of Antarctic ozone depletion. Antarctic Science, **3**, 3–11.

KARENTZ, D. and LUTZE, L. H. (1990): Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments. Limnol. Oceanogr., **35**, 549–561.

KARENTZ, D., CLEAVER, J. E. and MITCHELL, D. L. (1991): Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. J. Phycol., **27**, 326–341.

KORNMANN, P. (1955): Beobachtungen an *Phaeocystis*-kulturen. Helgoländer Wiss. Meeresunters., **5**, 218–233.

LEOBLICH, A. R. III and SMITH, V. E. (1968): Chloroplast pigments of the marine dinoflagellate *Gymnodinium resplendens*. Lipids, **3**, 3–15.

LORENZEN, C. J. (1979): Ultraviolet radiation and phytoplankton photosynthesis. Limnol. Oceanogr., **24**, 1117–1120.

LUBIN, D., FREDERICK, J. E., BOOTH, C. R., LUCAS, T. and NEUSCHULER, D. (1989): Measurements of enhanced springtime ultraviolet radiation at Palmer Station Antarctica. Geophys. Res. Lett., **16**, 783–785.

MARCHANT, H. J. and DAVIDSON, A. T. (1991): Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. Proceedings of the International Conference on the Role of Polar Regions in Global Change, ed. by G. WELLER *et al.* Fairbanks, Geophysical Institute, 397-400.

MARCHANT, H. J., DAVIDSON, A. T. and KELLY, G. J. (1991): UV-B protecting pigments in the

marine alga *Phaeocystis pouchetii* from Antarctica. Mar. Biol., **109**, 391–395.

MASKE, H. (1984): Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake. J. Plankton Res., **6**, 351–357.

MITCHELL, D. L. and KARENTZ, D. (1990): Molecular and biological responses of Antarctic phytoplankton to ultraviolet radiation. Antarct. J. U. S., **25**, 174–175.

PRÉZELIN, B. B. and SMITH, R. C. (1993): Polythene bags and solar radiation: Response. Science, **259**, 534–535.

SCHINDLER, D. W., SCHMIDT, R. V. and RIED, R. A. (1972): Acidification and bubbling as an alternative to filtration in determining phytoplankton production by the ^{14}C method. J. Fish. Res. Board Can., **29**, 1627–1631.

SMITH, R. C. and BAKER, K. S. (1989): Stratospheric ozone, middle ultraviolet radiation and phytoplankton productivity. Oceanography, **2**, 4–10.

SMITH, R. C., PRÉZELIN, B. B., BAKER, K. S., BIDIGARE, R. R., BOUCHER, N. P., COLEY, T., KARENTZ, D., MACINTYRE, S., MATLICK, H. A., MENZIES, D., ONDRUSEK, M., WAN, Z. AND WATERS, K. J. (1992): Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters. Science, **255**, 952–959.

SMITH, W. O., Jr. and NELSON, D. M. (1986): Importance of ice edge phytoplankton production in the Southern Ocean. BioScience, **36**, 251–257.

STOLARSKI, R. S., KRUEGER, A. J., SCHOEBERL, M. R., MCPETERS, R. D., NEWMAN, P. A. and ALPERT, J. C. (1986): Nimbus 7 satellite measurements of the springtime Antarctic ozone decrease. Nature, **322**, 808–811.

THOMSON, B. E., WORREST, R. C. and VAN DYKE, H. (1980): The growth response of an estuarine diatom (*Melosira nummuloides* [Dillw.] Ag.) to UV-B (290–320 nm) radiation. Estuaries, **3**, 69–72.

THOMSON, P. A., HARRISON, P. J. and PARSLOW, J. S. (1991): Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J. Phycol., **27**, 351–360.

TRODAHL, H. J. and BUCKLEY, R. G. (1989): Ultraviolet levels under sea ice during the Antarctic spring. Science, **245**, 194–195.

VELDHUIS, M. J. W., COLIJN, F. and VENEKAMP, L. A. H. (1986): The spring bloom of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. Neth. J. Sea Res., **20**, 37–48.

VERITY, P. G., VILLAREAL, T. A. and SMAYDA, T. J. (1988): Ecological investigations of blooms of *Phaeocystis pouchetii*-1. Abundance, biochemical composition and metabolic rates. J. Plankton Res., **10**, 219–248.

VETH, C. (1991): The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia-Weddell Sea. J. Mar. Sys., **2**, 451–464.

VOSJAN, J. H., DÖHLER, G. and NIEUWLAND, G. (1990): Effect of UV-B irradiance on the ATP content of microorganisms of the Weddell Sea (Antarctica). Neth. J. Sea Res., **25**, 391–393.

VOYTEK, M. A. (1989): Ominous future under the ozone hole: Assessing biological impacts in Antarctica. Washington, Environmental Defence Fund, 1–69.

WORREST, R. C. (1983): Impact of solar ultraviolet-B radiation (290–320 nm) upon marine microalgae. Physiol. Plant., **58**, 428–434.

WORREST, R. C. (1986): The effect of solar UV-B radiation on aquatic systems: An overview. Effects of Changes in Stratospheric Ozone and Global Climate, ed. by J. G. TITUS. 175–199.

WORREST, R. C., THOMSON, B. E. and VAN DYKE, H. (1981): Impact of UV-B radiation upon estuarine microcosms. Phytochem. Phytobiol., **33**, 861–867.

ZAR, J. H. (1984): Biostastical analysis. 2nd ed. New Jersey, Prentice-Hall, 653 p.

(Received May 6, 1993; Revised manuscript received September 7, 1993)

Proc. NIPR Symp. Polar Biol., 7, 70–81, 1994

MARKED INVERSE DISTRIBUTION OF SALPS TO OTHER MACROZOOPLANKTON IN WATERS ADJACENT TO THE SOUTH SHETLAND ISLANDS

Akito KAWAMURA, Kayoko MICHIMORI[1] and Jyunko MOTO[2]

Faculty of Bioresources, Mie University, 1515, Kamihama, Tsu 514

Abstract: Spatial distributions of salps and other zooplankton in the waters around the South Shetland Islands were examined. Two salp species, *Salpa thompsoni* and *Ihlea racovitzai*, were identified, and occurred with the latter more dominant than the former. At stations where salps dominated in terms of numerical abundance, other zooplankton were extremely scarce. Numerically, salps showed marked inverse distribution to copepods, amphipods, chaetognaths and polychaetes. This inverse distribution pattern covered the wide surface ranges of approximately 110×10^3 km^2. Its cause cannot be explained by either behavioral functions of organisms such as swarm formations or ecological regime such as interspecific exclusion. Predation by salps on zooplankton is also unlikely because only diatoms were found in the digestive tracts of salps. Salps inhabited waters with higher chl-*a* concentration than other zooplankton, and the contours of 0.3 mg·m^{-3} chl-*a* concentration corresponded roughly with the limit of salp distribution. This zonation by chl-*a* may be related to the distribution of Antarctic Winter Water (AWW), the subsurface temperature minimum. Higher chl-*a* together with low temperatures in the water column corresponded to the Antarctic Peninsula shelf water. Marked inverse distribution of salps and other zooplankton might be induced by an unknown function of the AWW.

1. Introduction

Many studies on macro- and mesozooplankton distributions in the Southern Ocean have been reported elsewhere, especially during and after the BIOMASS Program. From the community component point of view, however, the bulk of the past studies treated mainly copepods, euphausiids and other crustaceans due to their relative dominance and ecological importance in the zooplankton community. Except for some zoogeographical and taxonomical studies (APSTEIN, 1906; FOXTON, 1961, 1971; CASARETO and NEMOTO, 1986, 1987), salps in the Southern Ocean have been one of the less studied organisms. Recently, WIEBE *et al.* (1979) reported the important role of salps in the North Atlantic marine ecosystem.

In the Southern Ocean, occurrence of salps is usually occasional. Of eighty-two N70V net stations throughout the DISCOVERY investigations, '*Salpa fusifor-*

Present addresses:
[1]Environment Science Laboratory, Wakatsuru-cho, Kita-ku, Nagoya 464.
[2]Sanyo Techno-Marine, 3–6, Hichikenya, Higashi-Osaka 577.

ULTRAVIOLET RADIATION IN ANTARCTICA: MEASUREMENTS AND BIOLOGICAL EFFECTS
ANTARCTIC RESEARCH SERIES, VOLUME 62, PAGES 187-205

THE IMPACT OF ULTRAVIOLET RADIATION ON *PHAEOCYSTIS* AND SELECTED SPECIES OF ANTARCTIC MARINE DIATOMS

A. T. Davidson and H. J. Marchant

Australian Antarctic Division, Kingston, Tasmania 7050, Australia

Production of ultraviolet-absorbing compounds, survival, and growth rate of the prymnesiophyte *Phaeocystis pouchetii* and selected species of Antarctic diatoms after irradiation were investigated in laboratory experiments. In situ growth and primary production during irradiation and survival and growth after exposure were measured in Antarctic coastal waters. The colonial stage in the life cycle of *Phaeocystis* was found to contain high concentrations of ultraviolet-B radiation (UV-B) absorbing compounds which provided protection from UV irradiation. In contrast, the diatoms contained low concentrations of these compounds but survived higher UV-B irradiances than colonial *Phaeocystis*. Diatoms appear to use other mechanisms to reduce UV-B-induced mortality. Near-surface in situ incubations during February 1992 showed that the flagellate stage of *Phaeocystis* was the only alga examined which suffered severe mortality as a result of natural UV irradiances but that UV-A was responsible for most of this mortality. At sublethal irradiances, interspecific differences in production, in situ growth, and growth after irradiation were observed. Under the lower UV intensities experienced in situ these differences could lead to changes in phytoplankton species composition.

INTRODUCTION

Marked depletion of stratospheric ozone concentration has been observed between September and November since the mid-1970s [*Stolarski et al.*, 1986]. As a consequence of this depletion over Antarctica, the incident ultraviolet-B radiation (UV-B, 280-320 nm) in spring is at least as high as that at the summer solstice [*Frederick and Snell*, 1988; *Lubin et al.*, 1989]. That these wavelengths are known to be damaging to a range of organisms has lead to a surge of scientific interest to determine the UV climate of Antarctica and the impacts of UV irradiation on Antarctic organisms.

Phytoplankton form the base of the Antarctic food web and sustain the wealth of life for which the Southern Ocean is renowned [*Ainley et al.*, 1986]. Phytoplankton blooms in the sea ice and marginal ice zone (MIZ) are major contributors to phytoplanktonic production in Antarctica, but both appear susceptible to UV-B radiation. Sea ice algae contribute 10-50% of the primary production in some areas during spring [*Voytek*, 1989], but at this time of year the ice may be sufficiently transparent to UV that biologically significant doses are received by the ice algal community [*Trodahl and Buckley*, 1989; *Ryan*, 1992]. As the sea ice melts and retreats southward during spring and summer, it generates a shallow mixed depth in the MIZ. The high-light and high-nutrient waters of the MIZ support between 25 and 67% of the phytoplanktonic production in the Southern Ocean [*Smith and Nelson*, 1986]. Mixed depths during these blooms may be 20 m or less for up to 6 days [*Mitchell and Holm-Hansen*, 1991; *Veth*, 1991]. UV-B may penetrate to depths in excess 50 m in Antarctic waters [*Gieskes and Kraay*, 1990; *Karentz and Lutze*, 1990; *Smith et al.*, 1992] and reportedly reduces survival in the upper 10 m [*Karentz*, 1989] and photosynthesis in the upper 10-15 m [*Holm-Hansen et al.*, 1989]. *Smith et al.* [1992] found ozone depletion resulted in inhibition of integrated water column photosynthesis by at least 6-12%. Thus elevated UV-B irradiances resulting from the springtime ozone depletion and the subsequent breakup of the polar vortex coincide with the phytoplankton bloom in the ice and MIZ.

UV-B influences the growth, survival, life cycle, and species composition of many phytoplankton communities [*Lorenzen*, 1979; *Calkins and Thordardottir*, 1980; *Worrest et al.*, 1981; *Worrest*, 1983; *Jokiel and York*, 1984; *Maske*, 1984; *Hardy and Gucinski*, 1989; *Holm-*

Hansen et al., 1989; *Smith and Baker*, 1989; *Helbling et al.*, 1992]. In contrast, studies by *Gala and Giesy* [1991] and *Hobson and Hartley* [1983] found little inhibition of production by UV-B. Proposed scenarios concerning the future for Antarctic phytoplankton range widely. *El-Sayed et al.* [1990] concluded that Antarctic phytoplankton are UV stressed at present and are likely to be seriously affected by any increase in UV radiation. In Antarctic waters, *Holm-Hansen et al.* [1989] and *Cullen and Lesser* [1991] suggest that significant impacts would only be felt in the upper water column and that vertical mixing should ameliorate the impact of UV on phytoplankton.

Species and different life cycle stages differ in their growth and survival response to UV-B irradiance [*Calkins and Thordardottir*, 1980; *Karentz et al.*, 1991*a*; *Marchant et al.*, 1991]. It has been proposed that the most likely effect of elevated UV is a shift in the species composition of the phytoplankton favoring those species with greater tolerance [*Häder and Worrest*, 1991; *Karentz*, 1991; *Marchant and Davidson*, 1991]. To predict possible changes in phytoplankton composition and their ramifications for Antarctic marine ecosystems it is necessary to ascertain the UV photobiology of key species and the mechanisms they employ to enhance survival and production [*Karentz et al.*, 1991*a*].

Phaeocystis pouchetii is a cosmopolitan prymnesiophyte with two principal stages in its life cycle, free-swimming biflagellate cells, 5-8 μm in diameter, and a colonial form in which the cells are embedded in mucilage. These colonies can be more than 2 cm in diameter and contain thousands of cells [*Davidson and Marchant*, 1992*a*]. The colonial stage is among the most abundant and widespread organisms of the Antarctic marine ecosystem [*Fryxell and Kendrick*, 1988] and is one of the first species to bloom in the ice and in the top few meters of the MIZ [*Garrison et al.*, 1987; *Davidson and Marchant*, 1992*b*]. *Phaeocystis*, together with diatoms, principally of the genus *Nitzschia*, frequently dominates the phytoplankton of the ice edge bloom and plays a pivotal role in determining the structure of the planktonic community [*Garrison et al.*, 1987; *Fryxell and Kendrick*, 1988; *Garrison and Buck*, 1989; *Davidson and Marchant*, 1992*b*]. Any UV-mediated change in the abundance of *Phaeocystis* relative to diatoms would significantly alter the particle size, form, and availability of carbon to higher trophic levels and is likely to influence rates of vertical carbon flux [*Marchant and Davidson*, 1991]. In addition, it has been estimated that Antarctic *Phaeocystis* produces some 10% of the total biogenic dimethylsulfide (DMS) in the atmosphere [*Gibson et al.*, 1990]. DMS is known to be a principal source of sulfate cloud condensation nuclei and influence cloud cover and therefore global albedo [*Charlson et al.*, 1987; *Bates et al.*, 1987]. Thus any change in *Phaeocystis* productivity and abundance could influence DMS production. The impact of such a change is not known.

Numerous reviews address the impact of ozone depletion and the consequent increase in UV-B on phytoplankton [e.g., *Karentz*, 1990; *El-Sayed*, 1988; *Smith*, 1989; *Smith et al.*, 1992; *Smith and Baker*, 1989; *Voytek*, 1989, 1990; *Häder and Worrest*, 1991; *Karentz*, 1991]. It is not our intention to review the existing literature. Here we specifically address the effects of UV exposure on certain species of Antarctic phytoplankton and the possible ramifications of increased UV-B on the Antarctic ecosystem. Interspecific differences in UV tolerance have been reported [*Karentz et al.*, 1991*a*; *Calkins and Thordardottir*, 1980; *El-Sayed et al.*, 1990; *Karentz*, 1988; *Vosjan et al.*, 1990; *Worrest et al.*, 1981]. *Karentz* [1990] considers that interspecific difference in the ability to cope with UV may prove crucial in determining the ecological impact of elevated levels of UV-B, yet remarkably, little is known about the UV photobiology of key species of Antarctic marine phytoplankton. Here we outline a series of recent studies to address the question of the impact of UV on some key Antarctic phytoplankton.

METHODS

Laboratory Studies

Experiments designed to examine the photobiological responses of phytoplankton to UV exposure have varied considerably in their irradiance treatments [e.g., *Calkins and Thordardottir*, 1980; *Döhler*, 1984; *Cullen and Lesser*, 1991; *Karentz et al.*, 1991*a*]. Our experiments used UV-B exposures in the laboratory which approximate the irradiance that phytoplankton cells encounter in the natural environment [*Marchant et al.*, 1991; *Davidson et al.*, 1994] or are in situ incubations carried out in inshore waters near the Australian Antarctic station of Davis (68°30'S, 77°50'E). Laboratory and in situ incubations obviously cannot replicate the dynamic nature of the Antarctic marine ecosystem. The irradiance period was also relatively short as shallow mixed depth at the MIZ may remain for up to 6 days [*Veth*, 1991].

Seven species of the Antarctic diatom were isolated from Prydz Bay, Antarctica: *Chaetoceros simplex* Ostenfeld, *Nitzschia lecointei* V. H., *Nitzschia curta* (V. H.) Hasle, Proboscia (Rhizosolenia) alata (Brightwell) Sundström, *Proboscia (Rhizosolenia) inermis* (Castracane) Jordan and Ligowski, *Thalassiosira tumida*

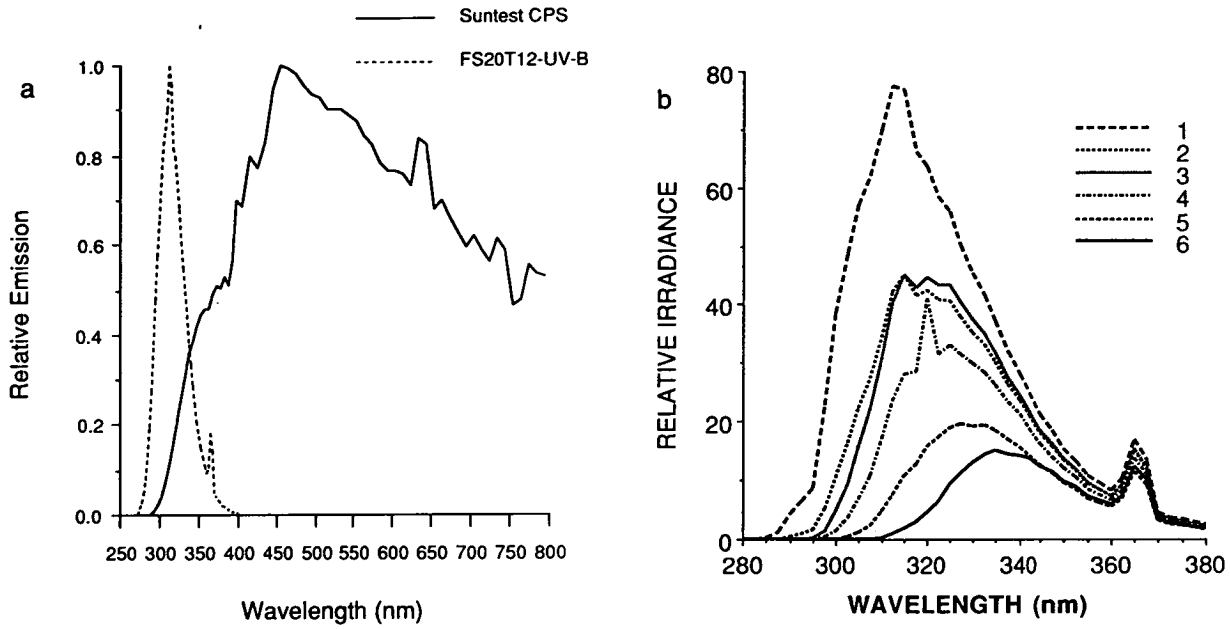


Fig. 1. Emission spectra of (*a*) Haraeus Suntest UV/white light source and FS20T12-UV-B Westinghouse sunlamps (redrawn from instrument specifications) and (*b*) spectral distribution experienced by algae in experiments with increasing UV-B only. Relative irradiance was calculated from relative emission (maximum emission = 1) of UV-B fluorescent tubes (Westinghouse instrument specifications) times spectrophotometric transmittance of attenuating screens used in the experiments including attenuation due to polystyrene culture flask. Curve 1 represents the polystyrene flask alone, and curves 2-6 represent increasing attenuation by glass screens. All experimental UV-B irradiances were obtained using a single UV-B fluorescent tube except the two highest irradiances (not plotted), which had spectral distributions 1 and 2 but required two UV-B tubes.

(Jan.) Hasle, *Stellarima (Coscinodiscus) microtrias* (Ehrenberg) Hasle and Sims. A predominantly flagellate and two monospecific (axenic) *Phaeocystis* cf. *pouchetii* (Hariot) Lagerheim were isolated from Prydz Bay, and *Phaeocystis* cultures of temperate origin (Commonwealth Scientific and Industrial Research Organization (CSIRO) Culture Collection of Microalgae, Hobart, Tasmania, and Plymouth Culture Collection, United Kingdom) were also used [*Marchant and Davidson*, 1991]. Antarctic and temperate *Phaeocystis* strains were maintained in GP5 medium [*Loeblich and Smith*, 1968] in glass flasks at 4° and 17°C, respectively, on a 12-hour light/12-hour dark cycle under cool white fluorescent tubes at an intensity of 6.19 ± 0.76 J m^{-2} s^{-1} with no UV-B enhancement. Antarctic diatoms were grown as above but in f/2 medium [*Guillard and Ryther*, 1962] and at an irradiance of 11.80 J m^{-2} s^{-1}.

Phaeocystis cultures in exponential growth phase were illuminated with either increasing total irradiance or increasing UV-B only (omitting the Antarctic flagellate strain). Diatoms were only exposed to increasing UV-B irradiances. An Haraeus Suntest CPS Xenon arc UV/white light source which closely reproduces the spectral composition of solar radiation (instrument specifications) was used to generate increasing total irradiance (Figure 1*a*). Cool white fluorescent tubes and FS20T12-UV-B Westinghouse sunlamps with peak emission at 313 nm (Figure 1*a*) gave photosynthetically available radiation (PAR) at 3.99 ± 1.00 x 10 W m^{-2} and UV-A at 0.70 ± 0.36 W m^{-2}. The spectral distribution and resulting intensity of UV-B was varied by attenuation with glass screens and the polystyrene culture flasks (Lux) in which the organisms were grown (Figure 1*b*). The irradiance to which the cells were exposed (beneath the glass screen and polystyrene) was measured with an International Light IL 1700 radiometer equipped with detectors to measure PAR, UV-A, and UV-B (Figure 2). Primary calibration of detector response was made using a National Institute of Standards and Technology intercomparison package (NIST test 534/240436-88) with further calibration using four International Light primary transfer standards.

An aliquot of 50 mL was removed from each strain of *Phaeocystis* at midexponential growth phase and was concentrated to 5 mL in the GP5 growth medium by centrifugation at 200 g for 50 min at their incubation temperature. The predominantly flagellate strain was filtered through 20-μm mesh netting to remove any

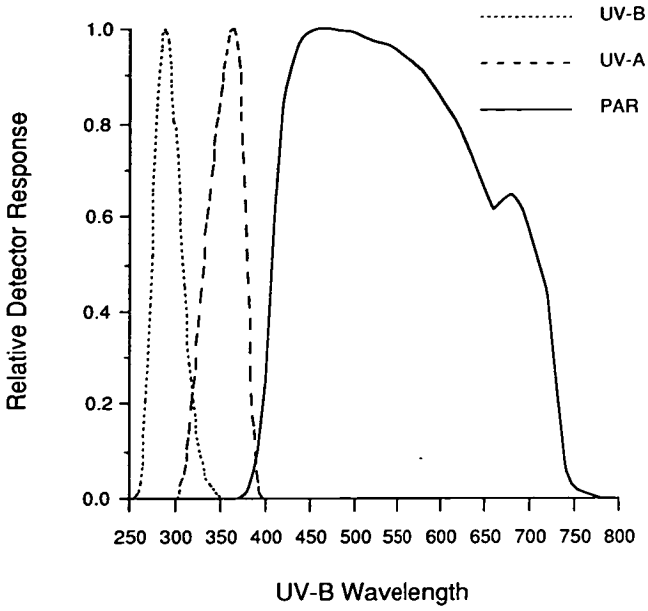


Fig. 2. The wavelength response of detectors used to measure PAR, UV-A, and UV-B (redrawn from instrument specifications).

colonies before the cells were similarly concentrated by centrifugation. Absorbance of the aqueous concentrates was measured with an Hitachi 3200 spectrophotometer. The GP5 medium exhibited no significant absorbance at UV or visible wavelengths before or after sustaining *Phaeocystis* growth. The absorbance of *Phaeocystis* concentrates was measured against a growth medium blank.

Axenic Antarctic colonial, axenic Antarctic flagellate, and temperate colonial strains were incubated at various UV irradiances for 48 hours under the 12-hour light/12-hour dark regime (i.e., 6-, 12- and 6-hour light exposures with intervening 12-hour dark periods). Aliquots of the cultures were fixed with Lugol's iodine and sedimented, and the percentage of undamaged cells was determined using a minimum of 300 cells over five replicate fields for each treatment. Control cultures were retained in culture maintenance conditions.

The long-term viability of irradiated cells was tested in four replicates of exponential growth phase cultures of Antarctic colonial *Phaeocystis*. These were exposed to irradiances spanning the UV-B irradiance range and the same illumination regime as used above together with an unirradiated control. A 30-mL aliquot from each treatment was added to 70 mL of fresh medium and the number of live cells in 15 replicate fields counted on days 0 and 13. Growth rates of all treatments were calculated [*Verity et al.*, 1988], and that of the unirradiated control was used to predict the initial concentration of viable cells in irradiated treatments from the observed final cell concentration.

Measurements of UV-B absorption were made using samples concentrated by centrifugation and extracted for 30 min at 50°C in growth medium [*Scherer et al.*, 1988]. This avoided problems associated with loss of *Phaeocystis* colonial material during filtration [e.g., *Lancelot*, 1984; *Veldhuis and Admiraal*, 1985; *Lancelot and Mathot*, 1987; *Verity and Smayda*, 1989]. It was established that 30 min was the extraction time at which there was maximum recovery of the UV-B-absorbing compounds. Absorbance was again measured against a growth medium blank, and peak height from these extractions was obtained by measuring the absorbance at 271 nm and 323 nm and subtracting the absorbance at these wavelengths from a line tangential to the absorbance minima around 250 nm and 380 nm. This removed the nonlabile background absorbance observed in Figure 4*b*.

Exponentially growing colonial *Phaeocystis* strains from Antarctica, Tasmanian coastal waters, East Australian Current, and the North Sea and English Channel were grown as for aqueous concentrates (i.e., without UV-B enhancement) and extracted at 50°C for 30 min in medium, and the 323-nm absorbance was calculated per unit chlorophyll-*a* concentration of the culture. Chlorophyll-*a* was extracted with methanol [*Wright and Shearer*, 1984], and its concentration calculated from the spectrophotometric equations of *Lorenzen* [1967].

After exposure of the temperate and Antarctic colonial cultures to various UV-B irradiances with constant PAR and UV-A (see above), UV-absorbing compounds were extracted at 50°C for 30 min in medium, and 323-nm absorption was calculated per live cell to ascertain whether exposure to UV-B influenced production of the UV-B-absorbing compounds. Three xenic and axenic Antarctic colonial strains were also extracted, and absorption at 323 nm per unit chlorophyll-*a* concentration was calculated (as above). Absorbance per unit chlorophyll-*a* was compared at 271 nm and 323 nm by paired *t* test.

Survival of diatoms was less easily determined by microscope than for *Phaeocystis*. Thus an extension of the calculation of *Marchant et al.* [1991] was employed to estimate viable cell concentration. Immediately after irradiation (day 0) a 5- or 10-mL aliquot (depending on cell concentration) of the control culture was sedimented with Lugol's iodine, and the concentration of cells with cytoplasmic contents (defined as living cells) was calculated from counts over 15 replicate fields, using an

inverted microscope. The mean cell concentration in the control culture at day 0 was then calculated ($N_{0\ \text{control}}$). Also on day 0, a 5-mL aliquot of each irradiated culture and the control was inoculated into 30 mL of f/2 medium in a glass flask and returned to the culture maintenance conditions described previously. These subcultures were incubated for up to 10 days, and the concentrations of live cells were counted at 2- to 4-day intervals depending on their growth rate. The growth rate of the control culture of each species (K_{control}) was calculated using the equation of *Verity et al.* [1988] (equation (1)). Equation (2) was then used to calculate the viable cell concentration on day 0 ($N_{0\ \text{irradiated}}$) from the growth rate of the control (K_{control}), for each of the 15 replicate cell concentrations for each species at each irradiance ($N_{t\ \text{irradiated}}$) and the time of culture ongrowth (t):

$$K = 1/t \times \log_2(N_t/N_0) \quad (1)$$

$$N_{0\ \text{irradiated}} = N_{t\ \text{irradiated}}/2^{K\ \text{control} \times t} \quad (2)$$

$$S\% = (N_{0\ \text{irradiated}}/N_{0\ \text{control}}) \times 100 \quad (3)$$

where K is growth rate, t is the number of days of growth, N_t is the number of cells at time t, N_0 is the number of cells immediately after irradiation (day 0), and $S\%$ is percent survival.

The viable cell concentration of each replicate field at day 0 ($N_{0\ \text{irradiated}}$) was then transformed to percent survival ($S\%$) in comparison with the unirradiated control at day 0 ($N_{0\ \text{control}}$) using (3). During calculation the survival of irradiated cultures was not allowed to exceed 100%. The percent survival in each replicate field was arcsine square root transformed, the mean and standard error of the replicate fields computed, and the mean, upper, and lower confidence intervals sine squared to revert the data to percentages [*Zar*, 1984].

Equation (1) was then used to calculate the growth rate of all ongrown irradiated cultures. Growth rates were calculated for each species using the day at which the cell concentration in culture had reached a sufficient concentration to allow statistically acceptable mean estimates (N_0) and that 2 days later (N_t). For each species, t_0 were as follows: *N. lecointei*, day 4; *P. alata*, day 6; *P. inermis*, day 6; *T. tumida*, day 8; and *S. microtrias*, day 8.

Absorption of cell extracts from controls and irradiated samples were measured. A known volume of culture was filtered through 25-mm-diameter Whatman GF/F filters. Filters were cut up into a glass homogenizer tube and 1.5 mL of 4:1 methanol:tetrahydrofuran (MeTHF) was added. The sample was then homogenized using a Teflon-headed grinder for 30 s at approximately 2000 rpm and decanted into a centrifuge tube. A further 0.5 mL of MeTHF was added to rinse the homogenizer; this was again decanted into the centrifuge tube, and the sample was centrifuged at 400*g* for 10 min at 0°C. The absorbance of the supernatant was measured using a Hewlett Packard 8450A spectrophotometer. If measurements were not carried out immediately, the extracts were stored at −120°C for no more than 4 weeks. The wavelength of maximum UV absorbance was identified and the peak absorption height measured as for *Phaeocystis*. Absorbance was then normalized against the chlorophyll-*a* peak height at 665 nm and calculated cell carbon (C) concentration and averaged over all cultures that received sublethal irradiances. Cell carbon content was calculated for each species from cell volume and the equations of *Epply et al.* [1970]. The absorbance of UV-absorbing compounds was expressed per unit cell C to allow comparison between species that varied in volume from around $7.90 \times 10^2\ \mu^3$ to $1.92 \times 10^5\ \mu^3$ for *N. lecointei* and *P. inermis*, respectively. UV absorbance was also normalized to cell concentration, and regression analysis of log absorbance per cell for each species was used to ascertain whether the concentration of UV-B-absorbing compounds was promoted by increased UV-B irradiance.

Absorption of MeTHF insoluble components of five diatom species was also measured using exponentially growing monospecific cultures of *N. lecointei*, *P. alata*, *T. tumida.*, *N. curta*, and *C. simplex*, grown in f/2 under culture maintenance conditions (as above). The methods employed aimed to cause as little damage to the insoluble frustule material as possible. A 75-mL aliquot of culture was centrifuged at 200*g* for 15 min at 0°C to concentrate the cells and the supernatant discarded. Two milliliters of 4:1 MeTHF was then added, the cells resuspended, and the intracellular pigments allowed to extract overnight at 0°C. The centrifugation was repeated and the absorption of the supernatant measured (as above). The remaining insoluble material was rinsed three times to remove any contamination from intracellular UV absorbing compounds by addition of 2 mL of MeTHF, the material resuspended and centrifuged at 200*g* for 10 min at 0°C, and the supernatant discarded. The material was then resuspended in a further 2.0 mL of MeTHF, and the absorbance measured as above.

In Situ Studies

Unialgal cultures of *C. simplex*, *S. microtrias*, *N. curta*, and *P. pouchetii* were maintained in culture as for laboratory cultures under cool white fluorescent light

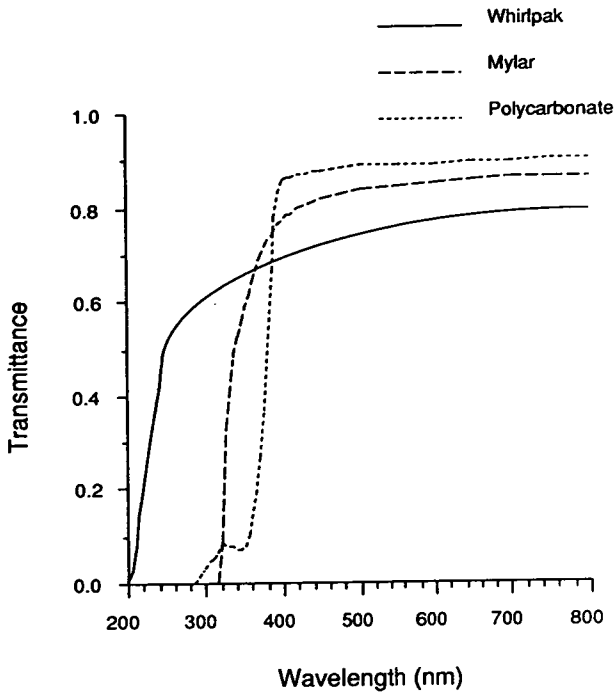


Fig. 3. The wavelength transmission of Whirlpak polythene bags, mylar, or polycarbonate UV screens used for generating different in situ light treatments.

but at PAR intensity of 5.11 J m^{-2} s^{-1}. Two hundred and fifty milliliters of exponentially growing cultures of each species was transferred to each of three Whirlpak bags which transmitted wavelengths above 220 nm (Figure 3). One bag remained unscreened while the remainder were screened with mylar (which transmitted wavelengths above 320 nm) or polycarbonate (which transmitted wavelengths longer than 370 nm) (Figure 3). Bags were then incubated at 0.3 m depth in near-shore waters off Davis between February 19 and February 26, 1992.

Primary production was estimated by ^{14}C bicarbonate incorporation. Fifty milliliters of the same culture of each species was transferred to Whirlpak bags for primary production, screened as above, and incubated in situ for 4 hours between 1030 and 1430 local solar time. Primary production by each species under each irradiance treatment was repeated after 2, 4, and 8 days irradiation as above. The light treatment of each primary production incubation was the same as that received during the 2-, 4-, and 8-day incubation. Lumagel was used as the scintillant, and counts were obtained with the using an LKB 1215 Rackbeta II liquid scintillation counter. Estimates of counting efficiency were performed each sample day before performing decay counts. Triplicate time zero blank and dark bag uptake were subtracted from counts in calculation of primary production.

Ten-milliliter subsamples of each exponentially growing culture were removed and fixed in Lugol's iodine for estimation of initial in situ cell concentration. Cell counts were performed as for laboratory cultures, and survival of each species under each light treatment was calculated (as for laboratory diatom cultures; see above). After 2, 4, and 8 days of in situ exposure, 10-mL subsamples were removed from each 250-mL Whirlpak, and in situ cell concentration was repeated as above. Five milliliters were was also inoculated into 30 mL of fresh medium, returned to culture maintenance conditions, and allowed to grow for estimation of survival and postirradiation growth. These cultures will henceforth be referred to as "postirradiation progeny."

The size of *P. pouchetii* flagellate and colonial cells was measured using an eyepiece micrometer in a Zeiss Photomicroscope II at 1000× magnification. A total of 200 cells were measured from each treatment which had been irradiated for 8 days and ongrown for a further 9 days.

RESULTS

Where possible, experimentation on monospecific culture strains of *Phaeocystis* and diatoms was conducted both in the laboratory and in situ. Laboratory studies examined the UV absorption characteristics, growth, and survival of species over irradiances sufficient to induce mortality. Maximum flux rates greatly exceeded that encountered in the natural environment. In situ studies addressed the growth, survival, and production responses of these species to naturally occurring UV flux rates.

UV-Absorbing Compounds

Aqueous concentrates of Antarctic colonial *Phaeocystis pouchetii* exhibited strong UV absorbance (Figure 4) [*Marchant et al.*, 1991]. Absorbance by the colony matrix (Figure 4, curve B), peaked at 211 nm. This absorption in the UV-C region of the spectrum would provide no significant protection from natural solar irradiation as these wavelengths are screened out by the atmosphere before reaching the Earth's surface. Much of the absorbance by the peak at 271 nm was also in the UV-C spectral region, but merging of the shoulder of this peak with that at 323 nm provided high absorbance between 250 and 370 nm (Figure 4, curve A).

The compounds with absorbance peaks at 271 and 323 nm were found to be water soluble and labile in the

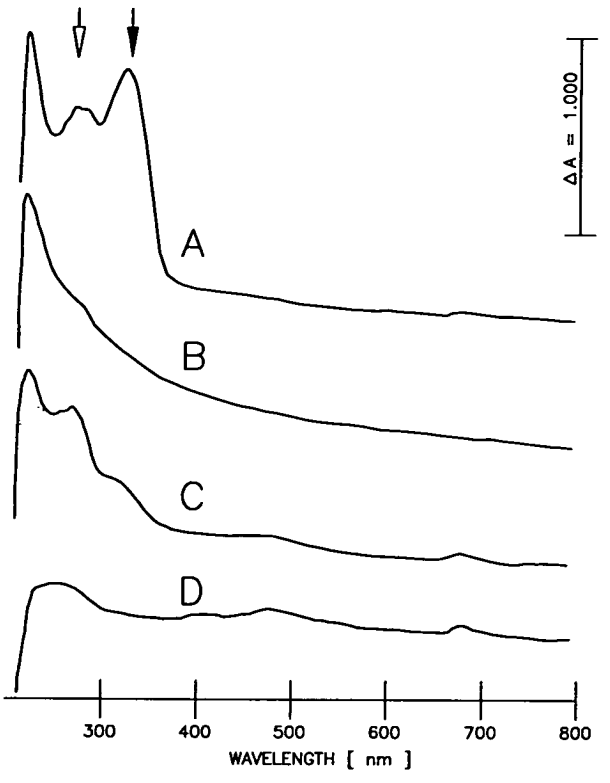


Fig. 4. Absorption spectra of aqueous *Phaeocystis* cell concentrates from colonies of axenic Antarctic isolate with prominent peak at 323 nm (solid arrow), shouldered peak at 271 nm (open arrow), unmarked peak at 211 nm, and peak at 680 nm from chlorophyll-*a* (curve A); mucilage of axenic colonies possessing only 211-nm peak (curve B); colonies from an East Australian Current isolate (curve C); and axenic Antarctic motile cells (curve D).

absence of bacteria [*Marchant et al.*, 1991]. Preliminary evidence indicates that the compounds comprise mycosporine-like amino acids (MAAs) and other as yet unidentified components (W. C. Dunlap, personal communication, 1992). Comparison of stationary growth phase cultures of axenic *Phaeocystis* strains with the same strains containing bacteria showed that the alga produces the UV-absorbing compounds and bacteria are involved in their decomposition [*Marchant et al.*, 1991]. Colonial *P. pouchetii* releases acrylic acid which acts as a broad spectrum bacteriocide [*Sieburth*, 1960] and inhibits bacterial growth in the vicinity of growing colonies [*Davidson and Marchant*, 1987]. Thus these UV-absorbing compounds would be afforded protection from bacterial decomposition within the colony. On the basis of the high concentration of UV-B absorbing compounds in Antarctic colonial *Phaeocystis* and the abundance in which this alga occurs in Antarctic waters, it has been suggested that this may provide some UV-B screening to organisms co-occurring with *Phaeocystis* [*Marchant et al.*, 1991]. Comparison of colonial strains from Antarctica, coastal Tasmania, the East Australian Current (EAC), and the English Channel showed that the Antarctic strain contained a 5-10 times greater concentration of UV-absorbing compounds than other strains (Figure 4, curve C) [*Marchant and Davidson*, 1991]. This indicates that the Antarctic strain of *Phaeocystis* cf. *pouchetii* is better screened against UV than those of other geographic origins. Why this should be, considering the relatively higher UV irradiance experienced at lower latitudes, is unclear.

Cells of the flagellate stage in the life cycle of *Phaeocystis* from Antarctica lacked any significant UV absorption (Figure 4, curve D).

TABLE 1. Wavelength of Peak MeTHF Soluble UV Absorbance, the Mean Ratio of UV-Absorbing Compound Peak Height to Chlorophyll-*a*, and the Absorbance per Microgram of Cell Carbon Calculated Over All Sublethal Irradiances

Species	Peak Absorbance, nm	MeTHF Soluble UV Abs:chl-*a*	MeTHF Soluble UV Abs/μg Cell C	Regression Statistic (Abs Versus Irradiance)	MeTHF Insoluble:Soluble UV Abs
N. lecointei	325	0.9	1.10×10^{-6}	$0.2 > P > 0.1$	4.7
P. alata	336	1.7	6.86×10^{-5}	$0.2 > P > 0.1$	3.1
P. inermis	340	2.1	6.17×10^{-5}	$0.5 > P > 0.2$	-
T. tumida	342	1.2	5.08×10^{-5}	$0.5 > P > 0.2$	0.75
S. microtrias	342	1.8	5.91×10^{-7}	$P > 0.50$	-
N. curta	342	1.1	-	-	6.6
C. simplex	342	1.0	-	-	9.44
P. pouchetii	323	27.	1.04×10^{-2}	$0.02 < P < 0.05$	-

Regression statistics were obtained in linear regression of MeTHF soluble UV absorbance against sublethal UV-B irradiance. The ratio of peak MeTHF soluble to insoluble UV absorbance is at the same wavelength.

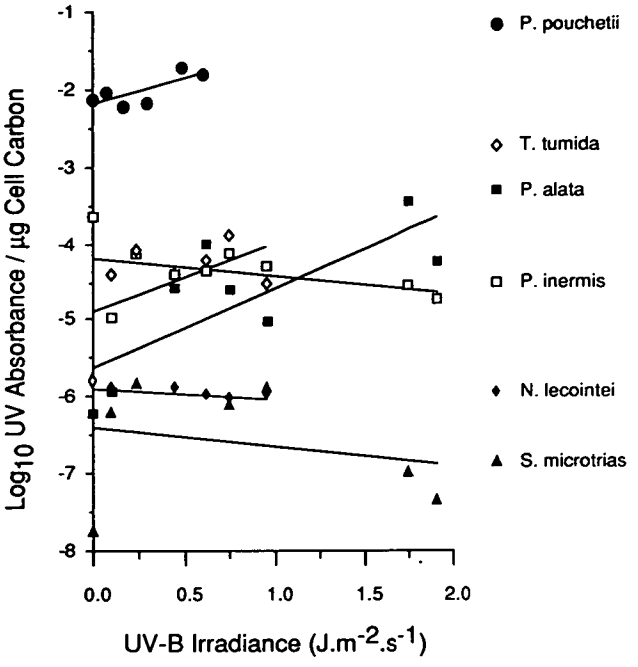


Fig. 5. Log UV absorbance per unit cell carbon of *P. inermis*, *N. lecointei*, *T. tumida*, *P. alata*, *S. microtrias*, and *Phaeocystis* plotted against UV-B irradiances.

The ratio of UV-B absorbance to chlorophyll-*a* for the colonial stage of *Phaeocystis*, 27.5:1, was more than an order of magnitude greater than that observed in any of the diatom species and the absorbance per microgram of cell carbon was in excess of between 2 and 4 orders of magnitude greater than that of the diatoms (Table 1). Colonial *Phaeocystis* demonstrated a substantial UV absorbance with no recent exposure to UV-B [*Marchant et al.*, 1991] and significantly increased the concentration of UV-absorbing compounds with increasing irradiance (Figure 5, Table 1). The high concentration of UV-absorbing compounds irrespective of the past light climate would protect against cell damage or death as a result of sudden exposure to UV. This would reduce the vulnerability of the remaining population as UV-B-absorbing pigment production by these cells would continue [*Marchant et al.* 1991]. However, with increasing UV-B irradiance, Antarctic *Phaeocystis* is able to further increase its protection. This contrasts with the EAC strain which, though possessing the 271- and 323-nm-absorbing compounds, was apparently incapable of increasing their concentration with increasing irradiance [*Marchant et al.*, 1991].

All species of diatom examined exhibited no absorbance peaks in the UV-B (Figures 6*a* and 6*b*, Table 1). Absorbance at these wavelengths was due to the shoulder of compounds with absorption peaks in the UV-A and UV-C region of the spectrum and background absorption which gradually increased with decreasing wavelength. None of the diatom species investigated showed high UV absorbance peaks (Table 1). The ratio of UV absorbance to that of chlorophyll-*a* for the five diatoms investigated ranged from 0.9 to 2.1:1, while the UV absorbance per microgram of cell carbon ranged from 5.91×10^{-7} to 6.86×10^{-5}. The diatom species were also exposed to constant UV-A and PAR irradiance and a range of UV-B irradiances. It was also found that increasing UV-B irradiance did not elicit any significant increase in the synthesis of UV-absorbing compounds (Table 1, Figure 5).

The low concentrations of UV-absorbing compounds, their absorbance peak outside the UV-B region, and the lack of any response in their synthesis to UV irradiance would indicate that these intracellular compounds are likely to provide little protection against UV-B irradiance. If these compounds are essential to cell metabolism, their absorbance in the UV-B may make them a target rather than a protective mechanism. Thus the significance of the UV-absorbing compounds in these diatom species remains uncertain.

Possession of intracellular UV-absorbing compounds by many diatoms may, however, be superfluous. Cells of five diatom species were carefully extracted in MeTHF, and the absorbance of the remaining insoluble material (consisting largely of frustule) was examined. This preliminary investigation showed that absorption in the UV region of the spectrum by the insoluble matter was around 3 to 10 times greater than the maximum MeTHF soluble absorption in the UV-B (Table 1) [*Davidson et al.*, 1994]. *T. tumida* was the only species that did not exhibit high absorption by MeTHF insoluble cell components. The reason for this is uncertain, but the large diameter and comparative lack of surface feature on the *T. tumida* frustule suggest that the amount of absorption may be due to size and the extent of surface ornamentation. Unlike the absorption by colonial *Phaeocystis* mucilage (Figure 4), that by the insoluble material in diatoms declined gradually with increasing wavelength and may provide substantial protection from UV radiation. Thus it is possible that possession of high concentrations of intracellular UV-absorbing compounds by *Phaeocystis* does not provide this species with a competitive advantage in environments which experience high UV flux rates. Rather, it increases its UV protection to a level similar to that possessed by diatoms and allows it to coexist in these environments.

Cell Survival and Growth

Calkins and Thordardottir [1980], *El-Sayed* [1988], *Karentz* [1990], *Karentz et al.* [1991*a*], and *Marchant and Davidson* [1991] have argued that the most likely effect of elevated UV radiation on Antarctic marine phytoplankton is a shift in the species composition. UV-induced changes in community composition have been observed in experimental systems [*Worrest et al.*, 1981; *El-Sayed et al.*, 1990]. *Calkins and Thordardottir* [1980] reported varying survival of marine diatom species isolated off Iceland, while *Karentz et al.* [1991*a*] demonstrated similar variability in diatoms from Antarctica and *Mitchell and Karentz* [1990] reported an almost 100-fold interspecific variation among Antarctic marine diatoms in the damage and repair of DNA at a given UV-B dose. These studies indicate that elevated UV-B levels may well act as a selective pressure to alter the species composition of Antarctic phytoplankton communities. Despite this, very few publications address the survival of individual phytoplankton species as a result of UV exposure.

Survival and growth in the laboratory. The occurrence of MAAs in marine organisms has received considerable attention because of their potential role in protecting these organisms against UV exposure [e.g., *Dunlap et al.*, 1988]. MAAs occur in many Antarctic marine organisms [*Karentz et al.*, 1991*b*], but it remains unknown whether their possession could enhance survival of these organisms when exposed to harmful UV-B wavelengths. Colonial *Phaeocystis* possess substantial concentrations of MAAs and other unidentified UV-absorbing compounds [*Marchant et al.*, 1991], while flagellate *Phaeocystis* and diatoms do not [*Davidson et al.*, 1994]. Thus comparison of the survival and growth of different life stages of *Phaeocystis* and between *Phaeocystis* and diatoms was undertaken to investigate the protection afforded to colonial *Phaeocystis* from UV-B by these compounds.

***Phaeocystis*:** Survival of Antarctic colonial *Phaeocystis* under increasing UV-B alone showed no significant decline in survival until UV-B irradiances exceeded 0.60 J m^{-2} s^{-1} with an irradiance resulting in 50% mortality (L.D. 50) around 0.85 J m^{-2} s^{-1} (Figure 7*a*). Under increasing simulated sunlight, survival declined at UV-B irradiances above 0.32 J m^{-2} s^{-1} with an L.D. 50 at approximately 0.50 J m^{-2} s^{-1} (Figure 7*b*). The initial decline in survival corresponds to around 60% peak and 150% average Antarctic surface UV-B irradiance [*Marchant et al.*, 1991]. Thus laboratory studies indicate that Antarctic colonial *Phaeocystis* cells

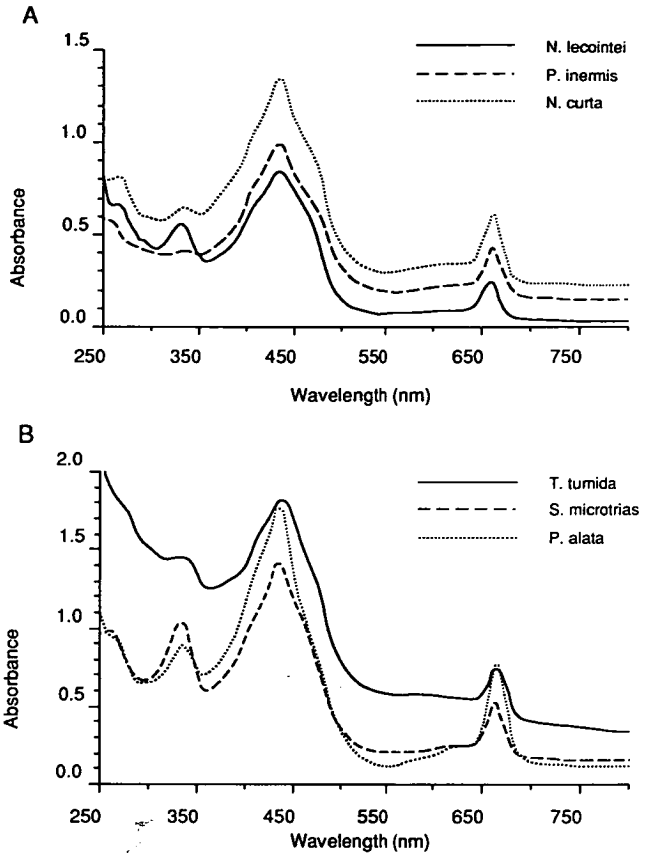


Fig. 6. Absorbance spectra of extracts in 4:1 methanol:tetrahydrofuran for cultures of (*a*) *N. lecointei*, *N. curta*, and *P. inermis* and (*b*) *T. tumida*, *S. microtrias*, and *P. alata* maintained without enhanced UV-B irradiances.

can withstand high UV-B but not sustained peak Antarctic surface irradiances. UV-B was not solely responsible for the observed mortality in the Antarctic colonial strain as high simulated solar irradiances (Figure 7*b*) resulted in lower survival at a given UV-B irradiance than increased UV-B alone (Figure 7*a*). In contrast, survival of EAC colonial *Phaeocystis* declined beyond the lowest irradiances, and survival at each UV-B irradiance was similar irrespective of the treatment (increasing simulated sunlight or UV-B only) (Figures 7*a* and 7*b*). The Antarctic flagellate stage (Figure 7*b*) also showed rapidly declining survival above the lowest irradiances.

Laboratory incubations demonstrate that flagellate cells from Antarctica which lacked UV-absorbing compounds were sensitive to UV-B irradiance. The EAC colonial *Phaeocystis* strain survived no better than Antarctic flagellate cells and is apparently afforded little protection by the low concentrations of UV-B-absorbing

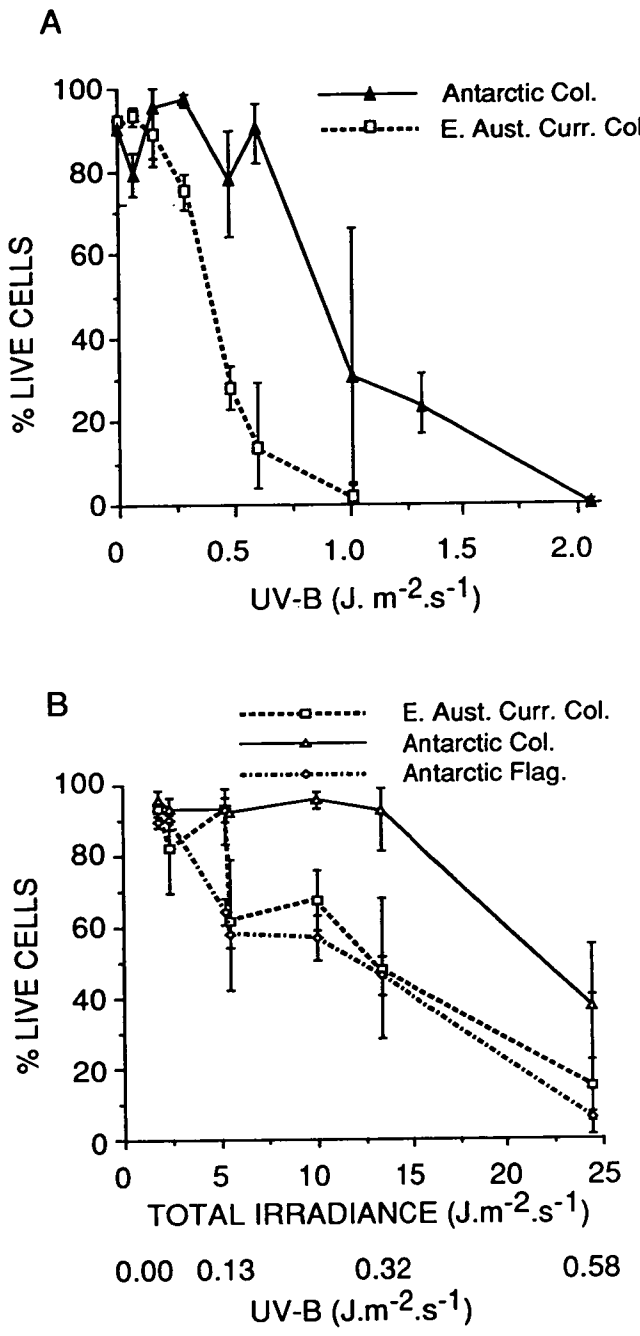


Fig. 7. Percentage of live *Phaeocystis* cells in (*a*) the East Australian Current colonial (E. Aust. Curr. Col.) and Antarctic colonial (Antarctic Col.) cultures exposed to increasing simulated solar irradiance provided by a Suntest UV/white light source and (*b*) Antarctic and East Australian Current colonial as well as Antarctic flagellate (Antarctic Flag.) cultures irradiated with various intensities of UV-B and constant PAR and UV-A. Error bars represent standard error calculated after *Zar* [1984].

compounds it possesses. In contrast, the Antarctic colonial *Phaeocystis* which contained high concentrations of UV compounds (Figure 7) had significantly higher survival ($P < 0.05$ for increasing UV-B alone and total radiation). Thus it appears that Antarctic colonial *Phaeocystis* is afforded substantial protection from UV-B-radiation by UV-absorbing compounds [*Marchant et al.*, 1991].

Diatoms: Five diatom species were exposed to increasing UV-B irradiances using methods similar to that employed for *Phaeocystis*. *Nitzschia lecointei* and *Proboscia alata* showed no significant decline until irradiances of 1.75 J m^{-2} s^{-1}, after which it fell to 0% at 3.40 J m^{-2} s^{-1} (Figures 8*a* and 8*b*). Survival of *Proboscia inermis*, *Thalassiosira tumida*, and *Stellarima microtrias* showed no significant decline until an irradiance of 3.40 J m^{-2} s^{-1} (Figures 8*c*, 8*d*, and 8*e*). Thus in comparison to survival of *Phaeocystis*, which declined to 30% at an irradiance of 1.0 J m^{-2} s^{-1} and had fallen to 0% at an irradiance of 2.1 J m^{-2} s^{-1} (Figure 8*f*), diatoms were able to withstand UV-B equivalent to between 3 and 6 times the present peak Antarctic surface irradiance. These diatoms exhibited a lack of intracellular UV-absorbing compounds and an inability to produce these compounds in response to increasing UV-B irradiance. Absorbance by cell components which are insoluble in MeTHF (Table 1) could largely compensate for this absence of intracellular compounds and, possibly in combination with other mechanisms, allow them to maintain the observed survival at remarkably high UV-B irradiances.

In situ survival and growth. PAR, UV-A, and UV-B have all been shown to elicit photoinhibition in phytoplankton [*Jones and Kok*, 1966]. High PAR irradiances are inhibitory to photosynthesis [*Bühlmann et al.*, 1987], but this is generally slight [*Jokiel and York*, 1984]. It is UV-A that is reported as being responsible for between 50 and 75% of the inhibition of photosynthesis and growth [e.g., *Jokiel and York*, 1984; *Maske*, 1984; *Holm-Hansen et al.*, 1989]. UV-B may contribute up to 25% of the photoinhibition experienced in near-surface waters [*Worrest*, 1983; *Helbling et al.*, 1992], but this inhibitory effect is only experienced in the upper 10-15 m [*Holm-Hansen et al.*, 1989]. UV-A irradiances penetrate to greater depths.

Phaeocystis: During in situ incubations the concentration of colonial *Phaeocystis* cells changed little in control cultures or those cultures which received UV-A or both UV-A and UV-B (Figure 9*a*). While all treatments apparently sustained no significant mortality, growth during incubation was slow or negligible and did not appear to change significantly as a result of changes

in irradiance. Exposure of colonial *Phaeocystis* to unscreened solar irradiance for periods of more than 2 days greatly increased the rate of growth of the postirradiance progeny (Figure 9*b*). Colonial cells which received solar irradiance with UV-B removed also showed a marked but lesser promotion of growth rate. The growth of control samples which were only exposed to PAR showed little increase in growth rate with incubation time. Thus it would appear that PAR, UV-A, and UV-B are inhibitory to growth during exposure but UV-A and UV-B may promote growth of some phytoplankton upon cessation of irradiation and return to maintenance conditions.

The concentration of flagellate cells clearly declined as a result of in situ UV radiation. Flagellate concentrations in the PAR-irradiated treatment remained approximately constant (Figure 9*c*). Cells in mylar screened treatments, which excluded UV-B, declined to around 20% of their original numbers over the 8-day period while unscreened flagellates declined at a similar rate but were almost absent after 8 days incubation. The growth of the flagellate stage postirradiance progeny exhibited similar trends in growth rate irrespective of irradiance treatment, suggesting that cells which survived the incubation were still viable. The only exception was the mylar-screened treatment after 4 days incubation (Figure 9*d*), the reasons for which are uncertain.

The cell diameter of colonial and flagellate stage cells increased under increasing UV-A and UV-B irradiance (Figures 10*a* and 10*b*). While the flagellate cell concentration fell with the introduction of UV-A and UV-B into the in situ incubation treatment (Figure 9*c*), the cell diameter of the flagellate stage increased (Figure 10*a*). This increase may reflect inhibition of division by UV irradiance, changes in flagellate cell stage [*Kornmann*, 1955; L. Peperzak, personal communication, 1993], or formation of flagellates from the colonial stage [*Veldhuis et al.*, 1986; *Verity et al.*, 1988; *Davidson and Marchant*, 1992*a*]. No significant differences in the concentration of colonial *Phaeocystis* cells resulted from removal of UV-A or UV-A and UV-B (Figure 9*a*). Thus the increase observed in the cell diameter of the colonial stage (Figure 10*b*) is not due to inhibition of division by UV, but instead is caused by other changes in cell physiology which also result in increased growth rates after return to culture maintenance conditions (Figure 9*b*).

Exposure to natural irradiances over a period of 8 days showed that survival in the postirradiance progeny of colonial Antarctic cells remained high for the entire period (Figure 11). The survival of the colonial stage of *Phaeocystis* is unaffected by exposure to in situ UV-A or UV-B irradiances. However, that of the flagellate

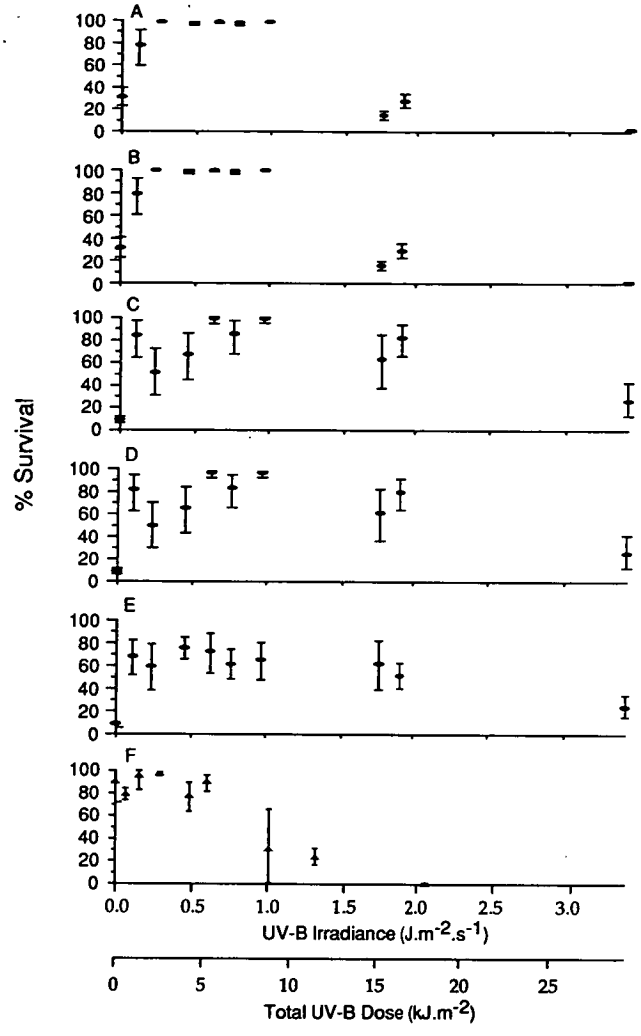


Fig. 8. Percent survival of (*a*) *N. lecointei*, (*b*) *P. alata*, (*c*) *P. inermis*, (*d*) *T. tumida*, (*e*) *S. microtrias*, and (*f*) *P. pouchetii* irradiated for 24 hours during 48 hours against UV-B irradiance. Error bars represent standard error calculated after *Zar* [1984].

stage showed a rapid decline in survival after 4 days exposure. Despite suffering significant mortality between day 2 and day 4 of in situ incubation (Figure 9*c*), those flagellate cells remaining appear viable (Figure 11). Irradiances between day 4 and day 8 remained relatively high, and by day 8 a marked decline in survival was observed. This decline was greatest when cultures were exposed to the total solar irradiance, but a major decline was also observed under a mylar screen which removed UV-B from the irradiance. Thus the majority of the observed mortality of the flagellate stage was due to UV-A radiation.

Diatoms: The diatoms *Stellarima microtrias*, *Chaetoceros simplex*, and *Nitzschia curta* were exposed

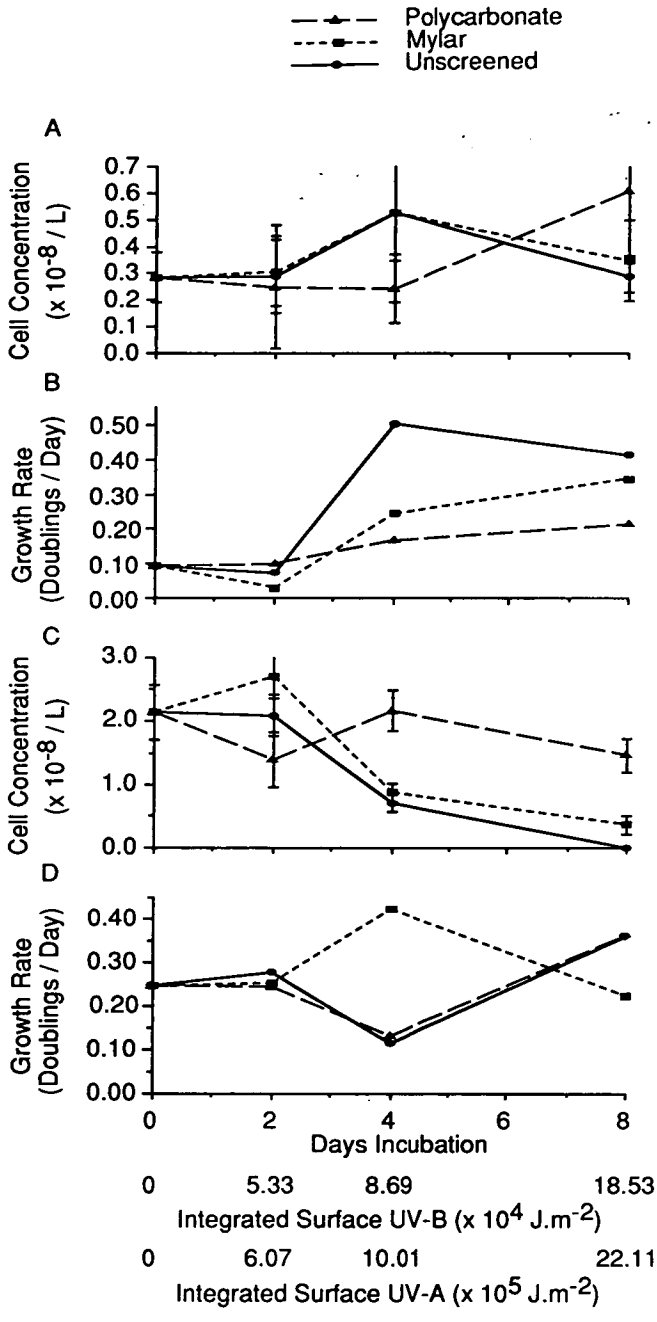


Fig. 9. Cell concentration during in situ incubation of (*a*) colonial and (*c*) flagellate Antarctic *Phaeocystis* cultures at an Antarctic coastal site and growth rate of (*b*) colonial and (*d*) flagellate cells subcultured from incubated cultures, returned to culture maintenance conditions, and allowed to grow for 9 days. Polycarbonate screens transmitted PAR only, mylar transmitted PAR and UV-A and unscreened treatments received full solar irradiance. Growth rate was calculated after *Verity et al.* [1988]. Total integrated UV-A and UV-B doses at each in situ sample period are given. Error bars represent standard deviation.

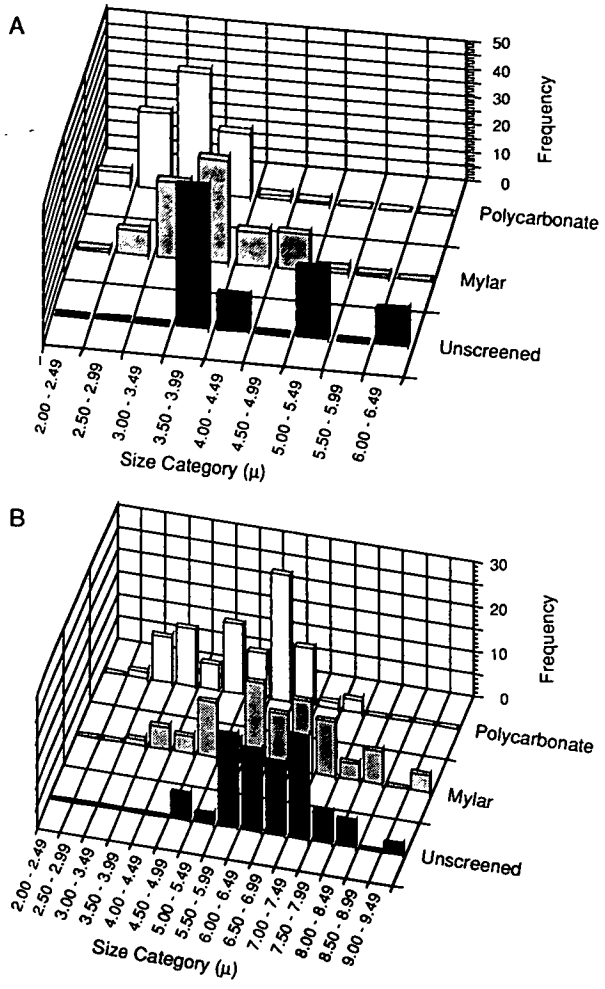


Fig. 10. Cell size distribution of the postirradiance progeny of (*a*) flagellate and (*b*) colonial cells incubated in situ at 0.3 m depth for 8 days beneath polycarbonate or mylar or unscreened, subcultured, and grown in culture maintenance conditions for 9 days.

to near-surface in situ irradiances in Antarctic coastal waters at the same time as *Phaeocystis*. Irrespective of irradiance treatment, the concentration of *C. simplex* and *S. microtrias* cells did not increase significantly during in situ irradiation (Figures 12*a* and 12*b*). The concentration of *N. curta* significantly increased in all treatments. The greatest increase was observed in the unscreened treatment during the first 4 days of irradiation, after which the concentration declined toward day 8 (Figure 12*c*), possibly as a result of the high irradiances during this time. This decline did not impact upon its survival (Table 2), indicating that surviving cells remained viable.

None of the species exhibited any significant decline in the survival over the 8-day irradiance as a result of

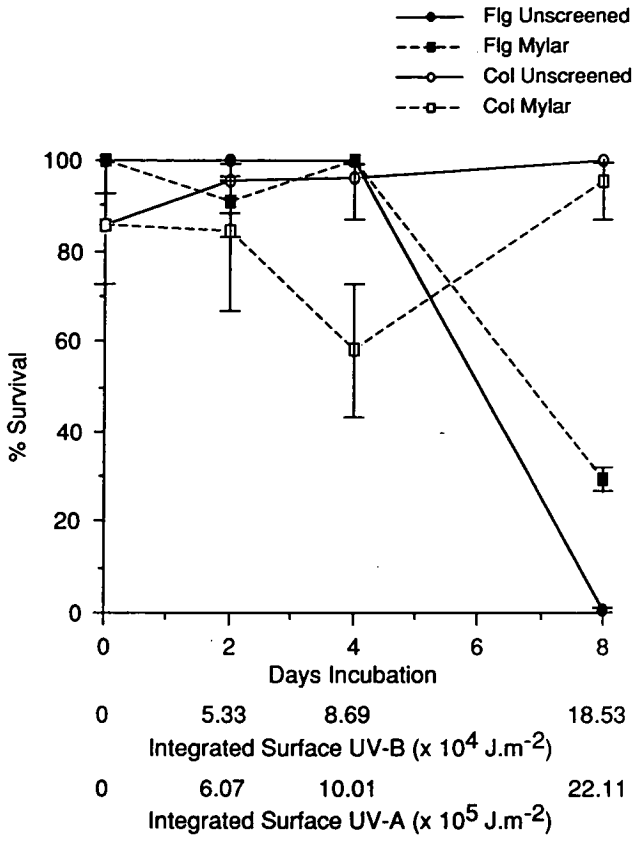


Fig. 11. Percent survival of unscreened (Flg unscreened) or mylar-screened (Flg mylar) flagellate stage and unscreened (Col unscreened) or mylar-screened (Col mylar) colonial stage Antarctic *Phaeocystis* culture during near-surface in situ incubations at Davis Station, Antarctica. Error bars represent standard error calculated after *Zar* [1984]

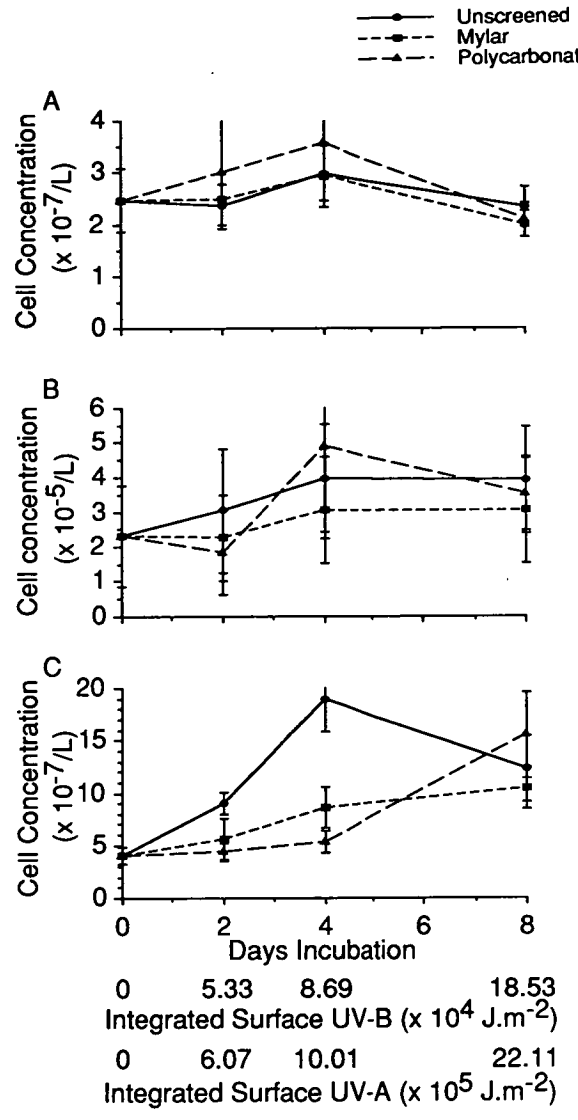


Fig. 12. Cell concentration of polycarbonate, mylar, and unscreened (*a*) *C. simplex*, (*b*) *S. microtrias*, and (*c*) *N. curta* during near-surface in situ incubations at Davis Station, Antarctica. Total integrated UV-A and UV-B doses at each in situ sample period are given. Error bars represent standard deviation.

UV (Table 2). However, the growth rate of subcultures of each species made after the 8 days in situ exposure did vary in response to the irradiance treatment received. *N. curta* showed a promotion of growth rate in the unscreened treatment similar to that observed for *Phaeocystis* (Table 3). The growth rate of the remaining species of diatoms declined with the progressive introduction of UV-A or UV-A and UV-B to the irradiance.

Diatom species exhibited differing growth responses to UV-B radiation. The photobiological strategy favored as a result of UV-B exposure would depend on the duration and intensity of the irradiance received. Though the diatoms we have examined survive high UV irradiances for a short time (Figure 11), their long-term survival and growth during and after irradiation may not advantage them over species that appear more vulnerable. For example, *S. microtrias* is able to survive UV-B intensities approximately an order of magnitude higher than that of *Phaeocystis*; however, it grows little better than *Phaeocystis* during in situ incubation, and irradiation with UV-B results in depression of growth after exposure. The growth rate of the postirradiance progeny for colonial *Phaeocystis* was approximately 3 times that of the PAR-irradiated culture, and this species may be favored at sublethal irradiances.

In situ primary production. UV is widely reported as being inhibitory to photosynthesis [e.g., *Lorenzen*, 1979; *Worrest et al.*, 1981; *Jokiel and York*, 1984; *Smith and Baker*, 1989; *Voytek*, 1989; *Häder and Worrest*, 1991].

TABLE 2. Percent Survival of Mylar or Unscreened Diatom Cultures Exposed to Near-Surface in Situ Irradiance for 8 Days Calculated After *Davidson et al.* [1994]

Species	No Screen			Mylar Screen		
	Mean	L1	L2	Mean	L1	L2
S. microtrias	95.88	99.87	86.77	93.53	98.55	85.28
C. simplex	99.17	99.97	96.04	98.59	99.91	95.70
N. curta	100.00	100.00	100.00	100.00	100.00	100.00

L1 and L2 represent upper and lower standard errors calculated after *Zar* [1984].

Estimates of inhibition by near-surface UV-B irradiances range from 15% to 30%, while UV-A resulted in a further decline of around 50% [*Maske*, 1984; *Holm-Hansen et al.*, 1989; *Helbling et al.*, 1992]. In Antarctic waters the increase in UV-B as a result of ozone depletion apparrently results in a reduction of at least 6-12 % in primary production [*Smith et al.*, 1992]. To our knowledge, however, no studies address simultaneously the production, survival, and growth for individual species as a result of UV irradiance.

We found inhibition of production was variable, probably as a result of variations in tolerance and photoadaptive ability of each species and changes in the in situ irradiance received. Photoinhibition was frequently greatest for treatments which received UV-B in the irradiance, but differences between light treatments were slight and percent inhibition seldom reached the magnitude reported above. However, we used nutrient-enriched monospecific cultures in our investigation, and the lower sensitivity may reflect the high-nutrient environment [*Cullen and Lesser*, 1991].

Phaeocystis: The photosynthetic rate of *Phaeocystis pouchetii* only declined slightly with incubation time, and little difference was observed between the irradiance treatments (Figure 13*a*). Fixation rates on day 4 may have been reduced by low surface irradiances. The carbon fixation rate per cell in the PAR-screened treatment also exhibited little change with time (Figure 13*b*); however, fixation rates per cell in the mylar and unscreened treatments increased rapidly as a result of the decrease in flagellate cell concentration (Figure 9*c*). Although no size-fractionated production was conducted to separate the flagellate and colonial stages, the colonial stage in the life cycle of this alga appears largely responsible for carbon fixation during these incubations as its numbers remained relatively constant during the 8 days of irradiation. In addition, the irradiance treatment and the flux rate during the production incubation appear to have little effect on the rate of production by the colonial stage (Figure 13*a*).

Diatoms: The diatom species investigated showed differing photosynthetic responses to the incubation (Figure 14). Primary production per cell of *Chaetoceros simplex* increased with time (Figure 14*a*) despite the cell

TABLE 3. Growth Rate of the Postirradiance Progeny of Diatoms After 8 Days Near-Surface in Situ Incubation Beneath Mylar or Polycarbonate or Unscreened, Subculturing and Grown in Culture Maintenance Conditions for 9 Days

Organism	Growth Rate		
	Polycarbonate	Mylar	Unscreened
S. microtrias	0.263	0.218	0.122
C. simplex	0.674	0.336	0.266
N. curta	0.253	0.289	0.657

Growth rate calculated after *Verity et al.* [1988]

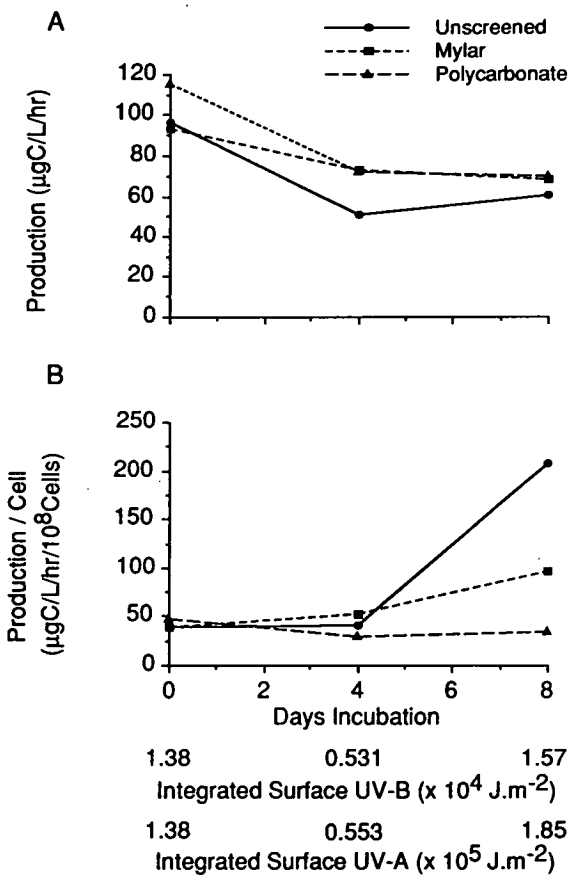


Fig. 13. The rate of (*a*) primary production and (*b*) production per cell by cultured *Phaeocystis* taken from near-surface in situ incubations at Davis Station, Antarctica. Fifty-milliliter subsamples were removed from polycarbonate, mylar, or unscreened light treatments and replaced in situ beneath the same screen for 4-hour incubations to estimate primary production. Surface UV-A and UV-B irradiance was integrated during each production incubation.

concentration during incubation remaining constant (Figure 12*a*). The rate of photosynthesis changed little with light treatment. Thus the differences between light treatments in growth of the postirradiance progeny (Table 3) must reflect UV-induced effects on processes other than photosynthesis. The rate of production per *Stellarima microtrias* cell declined slightly during the 8-day incubation though a slight increase may have resulted from the lower surface irradiances on day 4 (Figure 14*b*). The cell concentration did not change significantly over the duration of this experiment. Rates of production were consistently higher in mylar and polycarbonate-screened treatments. The observed differences in the growth rate of the postirradiance progeny (Table 3) may reflect differing photosynthetic reserves resulting from differences in rates of production.

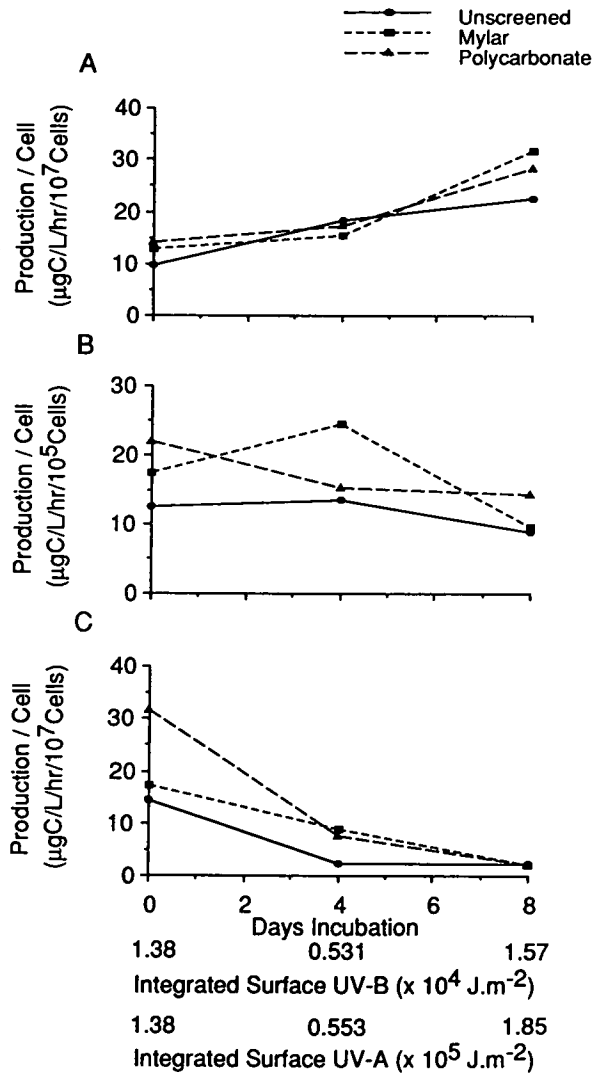


Fig. 14. The rate of primary production per cell by (*a*) *C. simplex*, (*b*) *S. microtrias*, and (*c*) *N. curta* during near-surface in situ incubations performed as for *Phaeocystis*.

In contrast with other diatoms, the per cell rate of production by *Nitzschia curta* fell (Figure 14*c*) while the cell concentration during the 8 day incubation period increased (Figure 12*c*). However, the division rate of the postirradiance progeny of the unscreened treatment for this species was more than twice that of other treatments (Table 3). This species appears capable of rapid recovery of photosynthetic ability after UV-B exposure. *Davidson et al.* [1994] suggest the possibility of UV-B being involved in repair of UV-A-related damage. This may explain the higher growth rate of the unscreened treatment than that receiving UV-A, but the reason for the lower growth rate of the postirradiance progeny exposed to PAR is unclear.

CONCLUSIONS

Considerable research has been devoted to the identification and quantitation of UV-absorbing compounds in a range of organisms from various environments [e.g., *Sivalingam et al.*, 1974; *Nakamura et al.*, 1982; *Dunlap and Chalker*, 1986; *Dunlap et al.*, 1986, 1988, 1989; *Scherer et al.*, 1988; *Carreto et al.*, 1990]. These compounds, which consist principally of mycosporine-like amino acids (MAAs), absorb biologically harmful UV wavelengths, are frequently possessed by organisms that inhabit high-UV environments, often alter their concentration in response to UV irradiation, and enhance the survival of organisms exposed to UV [*Dunlap et al.*, 1986, 1989; *Caldwell*, 1981; *Vernet et al.*, 1989; *Carreto et al.*, 1990; *Gieskes and Kraay*, 1990; *Karentz et al.*, 1991*b*; *Marchant et al.*, 1991]. It is unknown whether these compounds are synthesized with the primary function of protection from UV exposure, but it is widely accepted that these compounds provide a protective screen to shield cells from the full impact of ambient UV exposure and may constitute a significant protective strategy against UV damage [*Caldwell*, 1981; *Dunlap et al.*, 1986, 1989; *Carreto et al.*, 1990; *Karentz et al.*, 1991*b*].

A survey by *Karentz et al.* [1991*b*] of some 57 largely subtidal or intertidal species of Antarctic fish, invertebrates, and algae found that nearly 90% of those examined contained MAAs. Substantial levels of UV-absorbing substances have also been reported for natural assemblages of Antarctic plankton [*Mitchell et al.*, 1989; *Vernet et al.*, 1989; *Gieskes and Kraay*, 1990]. However, the number and identity of species possessing such compounds and their effectiveness as a UV screen remain unknown. Our results indicated that possession

of these pigments does confer a substantial protection to *Phaeocystis*. Considering also the reported absence or low concentration of these compounds in the diatoms [*Yentsch and Yentsch*, 1982; *Döhler*, 1984; J. Raymond personal communication, 1991], we tentatively suggested that *Phaeocystis* would increase its dominance at the expense of diatoms under the elevated levels of UV-B in Antarctica [*Marchant and Davidson*, 1991]. However, our laboratory studies indicate that these compounds do not endow *Phaeocystis* with greater survival than the diatoms which largely lack such protection [*Davidson et al.*, 1994]. UV absorption by the MeTHF insoluble material of many diatom species may contribute to the survival of diatom cells at UV-B irradiances greatly exceeding that of *Phaeocystis*.

The importance of the flagellate stage(s) in proliferation and dispersal of *Phaeocystis* in the Antarctic is unknown. In laboratory studies we found that the lethal UV intensity for *Phaeocystis*, particularly the flagellate stage, was lower than that of diatoms. In situ incubations of this species showed the flagellate cell stage suffered mortality as a result of near-surface UV radiation. However, UV-A was responsible for most of this mortality. As UV-A irradiances are not significantly enhanced as a result of ozone depletion, our results indicate that no major decline in *Phaeocystis* or diatom abundance as a result of UV-B-induced mortality is likely.

Data obtained from coastal waters near Davis Station during the 1988/1989 summer [*Davidson and Marchant*, 1992*b*] showed that the physiological peculiarities of the colonial stage of this alga, such as its release of dissolved organic carbon (DOC) and a bacteriocide, elicited the formation of a very distinct planktonic community. Microheterotrophic abundance was enhanced by high concentrations of *Phaeocystis*, while that of most other autotrophs and bacteria remained constant or declined. This contrasts with the community at the same site in 1991/1992, when *Phaeocystis* was present in much lower concentrations (F. Scott and H. J. Marchant, unpublished data, 1993). DOC concentrations were 4 orders of magnitude less than those in 1988/1989 and the microheterotroph concentrations were an order of magnitude less than that observed by *Davidson and Marchant* [1992*b*]. This reinforces our conclusion that *Phaeocystis* is a principal determinant of protistan abundance, species composition, and community function. *Phaeocystis* both is widespread and frequently dominates the Antarctic phytoplankton [e.g., *Garrison et al.*, 1987; *Fryxell and Kendrick*, 1988]. Any UV-induced decline in the abundance would substantially change their protistan characteristics and carbon dynamics in Antarctic waters [*Marchant and Davidson*, 1991].

Interspecific differences were also observed at sublethal UV irradiances during in situ incubations in Antarctic surface waters. *Smith et al.* [1992] found that UV-B irradiance in Antarctic waters beneath regions of ozone depletion caused a decline in productivity in the water column of between 6% and 12%. Compared with a naturally occurring interannual variability of around 25% [*Smith et al.*, 1988], the UV-B effect appears to be of little significance [*Smith et al.*, 1992]. This would appear reasonable were the suppression of production not species related. However, the decline in production observed by *Smith et al.* [1992] may be due to the differential inhibition of susceptible species. Comparison of *Chaetoceros socialis* and *Phaeocystis* sp. by *Smith et al.* [1992] demonstrated differing effect of irradiance on individual species. *Phaeocystis* showed significantly slower division rates under full solar irradiance than with UV-B removed, while the growth rate of *C. socialis* did not change significantly. This supported our results for *Phaeocystis* which demonstrate that, despite possessing UV-absorbing compounds, this species was more vulnerable to UV irradiance than the diatom species investigated [*Marchant et al.*, 1991; *Davidson et al.*, 1994; *Davidson and Marchant*, 1994].

The net effect of survival, UV-absorbing compound concentration, photosynthetic rate, and growth would determine the niche available to each species in the UV environment. The nature and duration of UV exposure in Antarctic waters have yet to be fully determined. The shallow blooms of the MIZ, which are responsible for much of the primary production in the Southern Ocean, appear vulnerable to increased UV-B radiation as a result of stratospheric ozone depletion [*Marchant and Davidson*, 1991]. The differing responses of the phytoplankton we have observed at lethal and sublethal irradiances support the suggestion that species or strains possessing greater tolerance to UV may be favored [*Häder and Worrest*, 1991; *Karentz*, 1991; *Marchant and Davidson*, 1991]. However, the interaction of in situ UV intensity, dose, and the photophysiology of each species is complex and the impact on the organisms is not great. The consequent changes in phytoplankton species composition may be sufficiently slow or slight that they are indiscernible from spatial and interannual variability.

Acknowledgments. We gratefully acknowledge Fiona Scott and Stephen Nicol for their comments on the manuscript and

Jeff Hunt, Paul Synnot, Lionel Whitehorn, Peter Sprunk, and Fiona Scott for their assistance with the field work.

REFERENCES

Ainley, D. G., W. R. Fraser, C. W. Sullivan, J. J. Torres, T. L. Hopkins, and W. O. Smith, Jr., Antarctic mesopelagic micronekton: Evidence from seabirds that pack ice affects community structure, *Science, 232*, 847-849, 1986.

Bates, T. S., R. J. Charlson, and R. H. Gammon, Evidence for the climatic role of marine biogenic sulphur, *Nature, 329*, 319-321, 1987.

Bühlmann, B., P. Bossard, and U. Uehlinger, The influence of longwave ultraviolet radiation (u.v.-A) on the photosynthetic activity (^{14}C-assimilation) of phytoplankton, *J. Plankton Res., 9*, 935-943, 1987.

Caldwell, M. M., Plant responses to solar ultraviolet radiation, in *Encyclopedia of Plant Physiology, New Series, Physiological Plant Ecology*, vol. 1, edited by O. L. Lange, P. S. Nobel, C. B. Osmond, and H. Ziegler, pp. 169-197, Springer, New York, 1981.

Calkins, J., and T. Thordardottir, The ecological significance of solar UV radiation on aquatic organisms, *Nature, 283*, 563-566, 1980.

Carreto, J. I., M. O. Carignan, G. Daleo, and S. De Marco, Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds?, *J. Plankton Res., 12*, 909-921, 1990.

Charlson, R. J., J. E. Lovelock, M. O. Andreae, and S. G. Warren, Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate, *Nature, 326*, 655-661, 1987.

Cullen, J. J., and M. P. Lesser, Inhibition of photosynthesis by ultraviolet radiation as a function of dose and dosage rate: Results for a marine diatom, *Mar. Biol., 111*, 183-190, 1991.

Davidson, A. T., and H. J. Marchant, Binding of manganese by Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release, *Mar. Biol., 95*, 481-487, 1987.

Davidson, A. T., and H. J. Marchant, The biology and ecology of *Phaeocystis* (Prymnesiophyceae), in *Progress in Phycological Research*, vol. 8, edited by F. E. Round and D. J. Chapman, pp. 1-45, Biopress, Bristol, England, 1992*a*.

Davidson, A. T., and H. J. Marchant, Protist interactions and carbon dynamics of a Phaeocystis-dominated bloom at an Antarctic coastal site, *Polar Biol., 12*, 387-395, 1992*b*.

Davidson, A. T., and H. J. Marchant, The in situ photobiology of Antarctic *Phaeocystis* and selected diatom species, *Polar Biol., 7*, 53-69, 1994.

Davidson, A. T., D. Bramich, H. J. Marchant, and A. McMinn, Effects of UV-B irradiation on growth and survival of Antarctic marine diatoms, *Mar. Biol.*, in press, 1994.

Döhler, G., Effect of UV-B radiation on the marine diatoms *Lauderia annulata* and *Thalassiosira rotula* grown in different salinities, *Mar. Biol., 83*, 247-253, 1984.

Dunlap, W. C., and B. E. Chalker, Identification and quantitation of near-UV absorbing compounds (S-320) in hermatypic scleractinian, *Coral Reefs, 5*, 15-159, 1986.

Dunlap, W. C., B. E. Chalker, and J. K. Oliver, Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia, III, UV-B absorbing compounds, *J. Exp. Mar. Biol. Ecol., 104*, 239-248, 1986.

Dunlap, W. C., B. E. Chalker, and W. M. Bandaranayake, New sunscreening agents derived from tropical marine organisms of the Great Barrier Reef, Australia, in *Proceedings of the 20th International Coral Reef Symposium*, vol. 3, edited by J. H. Choat et al., pp. 89-93, Sixth International Coral Reef Executive Committee, Townsville, Australia, 1988.

Dunlap, W. C., D. M. Williams, B. E. Chalker, and A. T. Banaszak, Biochemical photoadaptation in vision; UV-absorbing pigments in fish eye tissues, *Comp. Biochem. Physiol. B Comp. Biochem., 93*, 601-607, 1989.

El-Sayed, S. Z., Fragile life under the ozone hole, *Natur. Hist., 10*, 73-80, 1988.

El-Sayed, S. Z., F. C. Stephens, R. R. Bidigare, and M. E. Ondrusek, Effect of ultraviolet radiation on Antarctic marine phytoplankton, in *Antarctic Ecosystems: Ecological Change and Conservation*, edited by K. R. Kerry and G. Hempel, pp. 379-385, Springer-Verlag, New York, 1990.

Epply, R. W., F. M. H. Ried, and J. D. H. Strickland, Estimates of phytoplankton crop size, growth rate and primary production, in The Ecology of the Phytoplankton of La Jolla, California in the Period April Through September, 1967, edited by J. D. R. Strickland, *Bull. 17*, pp. 33-42, Scripps Inst. of Oceanogr., La Jolla, Calif., 1970.

Frederick, J. E., and H. E. Snell, Ultraviolet radiation levels during the Antarctic spring, *Science, 241*, 438-440, 1988.

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

Gala, W. R., and J. P. Giesy, Effects of ultraviolet radiation on the primary production of natural phytoplankton assemblages in Lake Michigan, *Ecotoxicol. Environ. Safety, 22*, 345-361, 1991.

Garrison, D. L., and K. R. Buck, The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsular regions, *Polar Biol., 10*, 211-219, 1989.

Garrison, D. L., K. R. Buck, and G. A. Fryxell, Algal assemblages in the Antarctic pack ice and in ice-edge plankton, *J. Phycol., 23*, 564-572, 1987.

Gibson, J. A. E., R. C. Garrick, H. R. Burton, and A. R. McTaggart, Dimethylsulfide and the alga *Phaeocystis pouchetii* in Antarctic coastal waters, *Mar. Biol., 104*, 339-346, 1990.

Gieskes, W. W. C, and G. W. Kraay, Transmission of ultraviolet light in the Weddell Sea: report of the first measurements made in the Antarctic, *BIOMASS Newsl., 12*, 12-14, 1990.

Guillard, R. R. L., and J. H. Ryther, Studies of the marine plankton diatoms *Cyclotella nana* Hustedt and *Detonula*

confervaceae (Cleve) Gran, *Can. J. Microbiol., 8*, 229-239, 1962.

Häder, D.-P., and R. C. Worrest, Effects of enhanced solar ultraviolet radiation on aquatic ecosystems, *Photochem. Photobiol., 53*, 717-725, 1991.

Hardy, J., and H. Gucinski, Stratospheric ozone depletion: implications for marine ecosystems, *Oceanography, 2*, 18-21, 1989.

Helbling, E. W., V. Villafane, M. Ferrario, and O. Holm-Hansen, Impact of natural ultraviolet radiation on specific marine phytoplankton species, *Mar. Ecol. Prog. Ser., 80*, 89-100, 1992.

Hobson, L. A., and F. A. Hartley, Ultraviolet irradiance and primary production in a Vancouver Island fjord, British Columbia, Canada, *J. Plankton Res., 5*, 325-331, 1983.

Holm-Hansen, O., B. G. Mitchell, and M. Vernet, Ultraviolet radiation in Antarctic waters: Effects on rates of primary production, *Antarct. J. U. S., 24*, 177-178, 1989.

Jokiel, P. L., and R. H. York, Jr., Importance of ultraviolet radiation in photoinhibition of microalgal growth, *Limnol. Oceanogr., 29*, 192-199, 1984.

Jones, L. W., and B. Kok, Photoinhibition of chloroplast reactions, I, Kinetics and action spectrum, *Plant Physiol., 41*, 1037-1043, 1966.

Karentz, D., DNA repair mechanisms in Antarctic marine microorganisms, *Antarct. J. U. S., 23*, 114 - 115, 1988.

Karentz, D., Report on studies related to the ecological implications of ozone depletion on the Antarctic environment, *Antarct. J. U. S., 24*, 175-176, 1989.

Karentz, D., Ecological considerations of the Antarctic ozone hole in the marine environment, in Effects of Solar Ultraviolet Radiation on Biogeochemical Dynamics in Aquatic Environments, *Tech. Rep. WHOI-90-09*, edited by N. V. Blough and R. G. Zepp, pp. 137-140, Woods Hole Oceanogr. Inst., Woods Hole, Mass., 1990.

Karentz, D., Ecological considerations of Antarctic ozone depletion, *Antarct. Sci., 3*, 3-11, 1991.

Karentz, D., and L. H. Lutze, Evaluation of biologically harmful ultraviolet radiation in Antarctica with a biological dosimeter designed for aquatic environments, *Limnol. Oceanogr., 35*, 549-561, 1990.

Karentz, D., J. E. Cleaver, and D. L. Mitchell, Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation, *J. Phycol., 27*, 326-341, 1991*a*.

Karentz, D., F. S. McEuen, M. C. Land, and W. C. Dunlap, Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: Potential protection from ultraviolet exposure, *Mar. Biol., 108*, 157-166, 1991*b*.

Kornmann, P., Beobachtungen an *Phaeocystis*-kulturen, *Helgolaender Wiss. Meeresunters., 5*, 218-233, 1955.

Lancelot, C., Extracellular release of small and large molecules by phytoplankton in the southern bight of the North Sea, *Estuarine Coastal Shelf Sci., 18*, 65-77, 1984.

Lancelot, C., and S. Mathot, Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters, I, Phytoplankton activity and related parameters, *Mar. Ecol. Prog. Ser., 37*, 239-248, 1987.

Loeblich, A. R., III, and V. E. Smith, Chloroplast pigments of the marine dinoflagellate *Gymnodinium resplendens*, *Lipids, 3*, 3-15, 1968.

Lorenzen, C. J., Determination of chlorophyll *a* and phaeopigments: Spectrophotometric equations, *Limnol. Oceanogr., 12*, 343-347, 1967.

Lorenzen, C. J., Ultraviolet radiation and phytoplankton photosynthesis, *Limnol. Oceanogr., 24*, 1117-1120, 1979.

Lubin, D., J. E. Frederick, C. R. Booth, T. Lucas, and D. Neuschuler, Measurements of enhanced springtime ultraviolet radiation at Palmer Station, Antarctica, *Geophys. Res. Lett., 16*, 783-785, 1989.

Marchant, H. J., and A. T. Davidson, Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean, in *Proceedings of the International Conference on the Role of Polar Regions in Global Change*, edited by G. Weller, C. L. Wilson, and B. A. B. Severin, pp. 397-400, Geophysical Institute, Fairbanks, Alaska, 1991.

Marchant, H. J., A. T. Davidson, and G. J. Kelly, UV-B protecting pigments in the marine alga *Phaeocystis pouchetii* from Antarctica, *Mar. Biol., 109*, 391-395, 1991.

Maske, H., Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake, *J. Plankton Res., 6*, 351-357, 1984.

Mitchell, B. G., and O. Holm-Hansen, Observations and modelling of the Antarctic phytoplankton crop in relation to mixing depth, *Deep Sea Res., 38*, 981-1007, 1991.

Mitchell, B. G., M. Vernet, and O. Holm-Hansen, Ultraviolet light attenuation in Antarctic waters in relation to particulate absorption and photosynthesis, *Antarct. J. U. S., 24*, 179-181, 1989.

Mitchell, D. L., and D. Karentz, Molecular and biological responses of Antarctic phytoplankton to ultraviolet radiation, *Antarct. J. U. S., 25*, 174-175, 1990.

Nakamura, H., J. Kobayashi, and Y. Hirata, Separation of mycosporine-like amino acids in marine organisms using reverse-phase high-performance liquid chromatography, *J. Chromatogr., 250*, 113-118, 1982.

Ryan, K. G., UV radiation and photosynthetic production in Antarctic sea ice microalgae, *J. Photochem. Photobiol. B Biol., 13*, 235-240, 1992.

Scherer, S., T. W. Chen, and P. Böger, A new UV-A/B protecting pigment in the terrestrial cyanobacterium *Nostoc commune*, *Plant Physiol., 88*, 1055-1057, 1988.

Sieburth, J. M., Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters, *Science, 132*, 676-677, 1960.

Sivalingam, P. H., T. Ikawa, Y. Yokohama, and K. Nisizawa, Distribution of 334 UV-absorbing-substances in the algae, with special regard of its possible physiological roles, *Bot. Mar., 17*, 23-29, 1974.

Smith, R. C., Ozone, middle ultraviolet radiation and the aquatic environment, *Photochem. Photobiol., 50*, 459-469, 1989.

Smith, R. C., and K. S. Baker, Stratospheric ozone, middle

ultraviolet radiation and phytoplankton productivity, *Oceanography, 2*, 4-10, 1989.

Smith, R. C., B. B. Prezelin, K. S. Baker, R. R. Bidigare, N. P. Boucher, T. Coley, D. Karentz, S. MacIntyre, H. A. Matlick, D. Menzies, M. Ondrusek, Z. Wan, and K. J. Waters, Ozone depletion: Ultraviolet radiation and phytoplankton biology in Antarctic waters, *Science, 255*, 952-959, 1992.

Smith, W. O., Jr., and D. M. Nelson, Importance of ice edge phytoplankton production in the Southern Ocean, *BioScience, 36*, 251-257, 1986.

Smith, W. O., Jr., N. K. Keene, and J. C. Comiso, Interannual variability in estimated primary productivity of the Antarctic marginal ice edge zone, in *Antarctic Ocean and Resources Variability*, edited by D. Sahrhage, pp. 131-139, Springer-Verlag, New York, 1988.

Stolarski, R. S., A. J. Krueger, M. R. Schoeberl, R. D. McPeters, P. A. Newman, and J. C. Alpert, Nimbus 7 satellite measurements of the springtime Antarctic ozone decrease, *Nature, 322*, 808-811, 1986.

Trodahl, H. J., and R. G. Buckley, Ultraviolet levels under sea ice during the Antarctic spring, *Science, 245*, 194-195, 1989.

Veldhuis, M. J. W., and W. Admiraal, Transfer of photosynthetic products in gelatinous colonies of *Phaeocystis pouchetii* (Haptophyceae) and its effect on the measurement of excretion rate, *Mar. Ecol. Prog. Ser., 26*, 301-304, 1985.

Veldhuis, M. J. W., F. Colijn, and L. A. H. Venekamp, The spring bloom of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters, *Neth. J. Sea Res., 20*, 37-48, 1986.

Verity, P. G., and T. J. Smayda, Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): Ingestion, egg production and growth of nauplii, *Mar. Biol., 100*, 161-171, 1989.

Verity, P. G., T. A. Villareal, and T. J. Smayda, Ecological investigations of blooms of *Phaeocystis pouchetii*, 1, Abundance, biochemical composition and metabolic rates, *J. Plankton Res., 10*, 219-248, 1988.

Vernet, M., B. G. Mitchell, and O. Holm-Hansen, Ultraviolet radiation in Antarctic waters: Response of phytoplankton pigments, *Antarct. J. U. S., 24*, 181-183, 1989.

Veth, C., The evolution of the upper water layer in the marginal ice zone, austral spring 1988, Scotia-Weddell Sea, *J. Mar. Syst., 2*, 451-464, 1991.

Vosjan, J. H., G. Döhler, and G. Nieuwland, Effect of UV-B irradiance on the ATP content of microorganisms of the Weddell Sea (Antarctica), *Neth. J. Sea Res., 25*, 391-393, 1990.

Voytek, M. A., Ominous future under the ozone hole: Assessing biological impacts in Antarctica, report, Environ. Def. Fund, Washington, D. C., 1989.

Voytek, M. A., Addressing the biological effects of decreased ozone on the Antarctic environment, *Ambio, 19*, 52-61, 1990.

Worrest, R. C., Impact of solar ultraviolet-B radiation (290-320 nm) upon marine microalgae, *Physiol. Pl., 58*, 428-434, 1983.

Worrest, R. C., B. E. Thomson, and H. V. Dyke, Impact of UV-B radiation upon estuarine microcosms, *Photochem. Phytobiol., 33*, 861-867, 1981.

Wright, S. W., and J. D. Shearer, Rapid extraction and high performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton, *J. Chromatogr., 294*, 281-296, 1984.

Yentsch, C. S., and C. M. Yentsch, The attenuation of light by marine phytoplankton with special reference to the absorption of near-UV radiation, in *The Role of Solar Ultraviolet Radiation in Marine Ecosystems*, edited by J. Calkins, pp. 691-706, Plenum, New York, 1982.

Zar, J. H., *Biostastical Analysis*, 2nd ed., 653 pp., Prentice-Hall, Englewood Cliffs, N. J., 1984.

(Received November 18, 1992;
accepted January 29, 1993.)

Marine Biology 109, 391–395 (1991)

Marine Biology

UV-B protecting compounds in the marine alga *Phaeocystis pouchetii* from Antarctica

H. J. Marchant [1], A. T. Davidson [1] and G. J. Kelly [2]

[1] Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia
[2] Department of Biology, Queensland University of Technology, 2 George Street, Brisbane, Queensland 4001, Australia

Date of final manuscript acceptance: February 22, 1991. Communicated by G. F. Humphrey, Sydney

Abstract. *Phaeocystis pouchetii* (Hariot) Lagerheim is widely distributed in polar waters, and forms massive near-surface blooms in the marginal ice-edge zone around Antarctica during spring and summer. UV irradiance in the Antarctic marine environment is reportedly as high in October and November as in mid-summer due to stratospheric ozone depletion. Because of the location and timing of the *P. pouchetii* bloom, this prymnesiophyte will be exposed to high levels of UV-B (280 to 320 nm) radiation. Colourless water-soluble compounds, produced by the colonial stage in the life cycle of this alga, absorb strongly between 250 and 370 nm, with absorbance maxima at 271 and 323 nm. The concentration of these compounds in cultured *P. pouchetii* depends on the strain, stage in the life cycle, and presence of bacteria. As well as conferring substantial protection to this alga, these substances may also provide UV protection to other organisms present in the water column.

Introduction

The marked depletion of stratospheric ozone over Antarctica in early October leads to an extended period when incident UV radiation is similar that in mid-summer (Frederick and Snell 1988). Sea-ice around Antarctica commences the southward retreat from its maximum extent in late September (Jacka 1983). Increasing insolation at this time leads to proliferation of algae in the sea-ice and the marginal ice-edge zone where the development of a shallow pycnocline limits vertical mixing and promotes the formation of blooms high in the water column (Smith 1987). Solar UV-B radiation penetrates seawater to depths that are able to influence the growth of macrophytes and phytoplankton (Jerlov 1950, Calkins and Thordardottir 1980, Worrest 1983, Maske 1984, Hardy and Gucinski 1989, Smith and Baker 1989, Wood 1989). However, the impact of UV radiation on Antarctic marine phytoplankton is equivocal (Roberts 1989, Voytek 1990).

The cosmopolitan alga *Phaeocystis pouchetii* is one of the first organisms to bloom in the ice and in the upper 10 m of the water column following the breakup of the sea-ice (Garrison et al. 1987, Fryxell and Kendrick 1988). This prymnesiophyte has two principal stages in its life cycle, free swimming biflagellate cells and a colonial stage in which the cells are embedded in mucilage. The colonial form of this alga is among the most abundant and widespread organisms of the Antarctic marine ecosystem (Fryxell and Kendrick 1988, Vincent 1988).

Many organisms inhabiting environments that are subjected to high incident UV radiation produce UV-B absorbing compounds to shield them from exposure to these wavelengths (Dunlap et al. 1986, 1989, Caldwell 1981, Wood 1989, Carreto et al. 1990). Here we report the presence of UV-B absorbing substances in *Phaeocystis pouchetii* and demonstrate in culture that they mitigate UV damage to this alga.

Materials and methods

A predominantly flagellate (Strain DE10) and two colonial axenic strains (A1–3 and A1–4) of *Phaeocystis pouchetii* (Hariot) Lagerheim, isolated from Prydz Bay, Antarctica (Antarctic Division Culture Collection, Hobart, Tasmania), and a unialgal strain (165-7), isolated from the East Australian Current (CSIRO Culture Collection of Micro-algae, Hobart, Tasmania) were maintained in GP5 medium (Loeblich and Smith 1968) in glass flasks at 4 and 20 °C, respectively on a 12 h light : 12 h dark cycle under cool-white fluorescent tubes at an intensity of 6.19 ± 0.76 W m^2 with no UV-B enhancement. An aliquot of 50 ml was removed from each strain at mid-exponential growth phase and was concentrated to 5 ml by centrifugation at $200 \times g$ for 50 min at their incubation temperature. The predominantly flagellate strain was first filtered through 20 μm mesh netting to remove any colonies before the cells were similarly concentrated by centrifugation. Absorbance of the aqueous concentrates was measured by monochromatic scanning using a Hitachi 3200 spectrophotometer.

The proportion of viable *Phaeocystis pouchetii* cells in Axenic Colonial A1–3, Axenic Flagellate DE10 and Colonial 165-7 strains was examined following exposure to enhanced levels of UV irradiation. Cultures in exponential growth phase were illuminated with either increasing total irradiance or increasing UV-B only (omitting

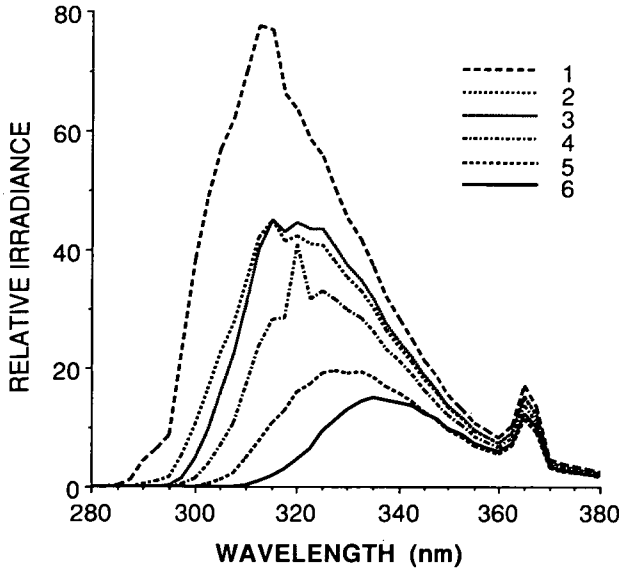


Fig. 1. Spectral distribution experienced by algae in experiments with increasing UV-B only. Relative irradiance was calculated from relative emission (maximum emission = 1) of UV-B fluorescent tubes (Westinghouse instrument specifications) × spectrophotometric transmittance of attenuating screens used in the experiments including attenuation due to polystyrene culture flask. Curve 1: polystyrene flask alone; Curves 2–6: increasing attenuation by glass screens. All experimental UV-B irradiances were obtained using a single UV-B fluorescent tube, except the two highest irradiances (not plotted) which had spectral distributions of 1 and 2 but required two UV-B tubes

Strain DE10). A Haraeus Suntest CPS Xenon arc UV/white-light source which closely reproduces the spectral composition of solar radiation (instrument specifications) was used to generate increasing total irradiance. Cool-white fluorescent tubes and FS20T12-UV-B Westinghouse Sunlamps gave photosynthetically active radiation (PAR) at $3.99 \pm 1.00 \times 10\ W\,m^2$, UV-A at $0.70 \pm 0.36\ W\,m^2$ while the spectral distribution and resulting intensity of UV-B was varied by attenuation with glass screens and the polystyrene culture flasks (Lux) in which the organisms were grown (Fig. 1). The irradiance to which the cells were exposed (beneath the glass screen and polystyrene) was measured with an International Light IL 1700 Radiometer equipped with detectors to measure photosynthetically active radiation (PAR), UV-A and UV-B. Primary calibration of detector response was made using a National Institute of Standards and Technology intercomparison package (NIST Test # 534/240436-88) with further calibration using four International Light primary-transfer standards. Control cultures were unirradiated. After incubation for 48 h under the 12 h light : 12 h dark regime (i.e., 6, 12 and 6 h light exposures with intervening 12 h dark periods) aliquots of the cultures were fixed with Lugol's iodine, sedimented, and the percentage of undamaged cells was determined using a minimum of 300 cells over five replicate fields for each treatment.

The long-term viability of irradiated cells was tested using four exponential growth phase cultures of Antarctic colonial *Phaeocystis pouchetii* (Strain A1–3), which were exposed to irradiances spanning the UV-B irradiance range under the same illumination regime as used above plus an unirradiated control. A 30 ml aliquot from each treatment was added to 70 ml of fresh medium and the number of live cells in 15 replicate fields was counted on Days 0 and 13. Growth rates of all treatments were calculated (Verity et al. 1988), and that of the unirradiated control was used to predict the initial concentration of viable cells in irradiated treatments from the observed final cell concentration.

Quantitative measurements of UV-B absorption were made using samples concentrated by centrifugation and extracted for 30 min at 50 °C in growth medium (Scherer et al. 1988). It was established that 30 min was the extraction time at which there was maximum recovery of the UV-B absorbing compounds. Peak height from these extractions was obtained by measuring the absorbance at 271 and 323 nm and substracting the absorbance at these wavelengths from a line tangential to the absorbance minima around 250 and 380 nm. This removed the nonlabile background absorbance observed in Fig. 2B.

Exponentially growing colonial *Phaeocystis pouchetii* strains from Antarctica (A1–3), Tasmanian coastal waters (PE.2, CSIRO Culture Collection of Micro-algae, Hobart, Tasmania), East Australian Current (165-7) and the North Sea and English Channel (Veldhuis and 540, Plymouth Culture Collection, England) were grown as for aqueous concentrates (i.e., with no UV-B enhancement), and were extracted at 50 °C for 30 min in the culture medium; the 323 nm absorbance was calculated per unit chlorophyll *a* concentration of the culture. Chlorophyll *a* was extracted with methanol (Wright and Shearer 1984) and its concentration was calculated from the spectrophotometric equations of Lorenzen (1967).

Colonial Cultures A1–3 and 165-7 were exposed to various UV-B irradiances with constant PAR and UV-A (see paragraph 2 above), and then extracted at 50 °C for 30 min in the culture medium; 323 nm absorption was calculated per live cell to ascertain whether exposure to UV-B influenced production of the UV-B-absorbing compounds.

Xenic and axenic Antarctic Colonial Strains A1–3, A1–4, DE10, RG1.2 were also extracted and absorption at 323 nm per unit chlorophyll *a* calculated. Absorbance per unit chlorophyll *a* was compared at 271 and 323 nm by a paired *t*-test. The value obtained for the A1–3 strain was also used to predict the attenuation of UV-B radiation in Antarctic coastal waters.

Results and discussion

UV-B absorbing substances are produced by a wide range of organisms, including the eye-lens tissue of fish (Dunlap et al. 1989), higher plants (Caldwell 1981), corals (Dunlap et al. 1986), algae (Sivalingam et al. 1974, Carreto et al. 1990) and cyanobacteria (Shibata 1969, Scherer et al. 1988). There is compelling evidence that such compounds shield the organisms from UV damage (Dunlap et al. 1989, Wood 1989, Carreto 1990).

Aqueous concentrates of the colonial stage of Antarctic strains of *Phaeocystis pouchetii* exhibit strong absorbance at 323 nm, a shouldered peak at 271 nm and a third peak at 211 nm (Fig. 2A). These absorbances greatly exceed that of chlorophyll *a* at 680 nm and carotenoids. The colourless UV-B absorbing compounds were fully soluble in water at 50 °C, as evidenced by the lack of any absorbance in resuspended concentrate from this extraction. These UV-B-absorbing compounds have also been found in field samples from Antarctic coastal waters (Davidson unpublished data).

Only the absorbance peak at 211 nm was present in aged axenic cultures in which no living cells remained (Fig. 2B). Thus, this peak represents the absorbance of the colony matrix which constitutes most of the algal volume (Davidson and Marchant 1987) and any residual material from dead cells. Absorbance at wavelengths shorter than 280 nm is unlikely to confer protection because of the marked attenuation of such short wavelengths by the atmosphere and water (Smith and Baker 1979), but provides a background absorbance on which is superimposed that of the protective compounds. The dis-

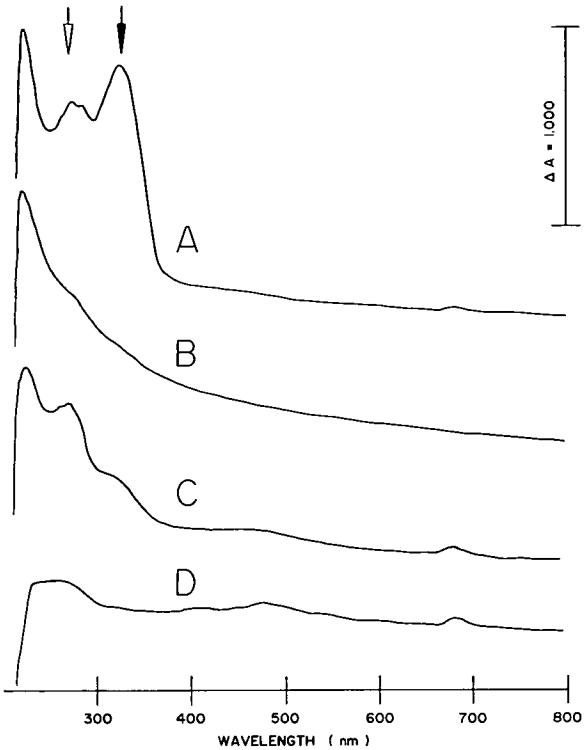


Fig. 2. *Phaeocystis pouchetii.* Absorption spectra of aqueous cell concentrates from (A) colonies of axenic Antarctic isolate with prominent peak at 323 nm (filled arrow), shouldered peak at 271 nm (open arrow), unmarked peak at 211 nm, and peak at 680 nm from chlorophyll *a*; (B) dead axenic colonies possessing only 211 nm peak; (C) colonies from an East Australian Current isolate; (D) axenic Antarctic motile cells

appearance of the 271 and 323 nm peaks from the aged axenic cultures shows that they are labile in the absence of bacteria. Absorption maxima were significantly higher at both 271 and 323 nm in late stationary-phase axenic cultures than those of the same strain and culture age containing bacteria ($0.025 < P < 0.050$, $n = 8$), indicating that the alga is producing the compounds and that bacteria are also involved in their decomposition. The ability of *Phaeocystis pouchetii* to inhibit bacterial growth within the immediate vicinty of growing colonies (Sieburth 1960, Davidson and Marchant 1987) such that their number, in the order of 10^8 cells l^{-1} (approximately an order of magnitude less than that during stationary phase) would prolong retention of the UV-B absorbing compounds by this life stage. That these compounds are labile and degraded by bacteria indicates that they are likely to be only short-lived outside growing colonies.

We have not been able to maintain *Phaeocystis pouchetii* from the East Australian Current in axenic culture, and thus bacterial decomposition of the UV-B absorbing compounds may contribute to the lower absorbance found in this strain (Fig. 2C). Bacterial impact was minimised, however, by using cultures in the exponential growth phase. Davidson and Marchant (1987) reported that bacterial numbers were more than an order of magnitude less in the mid-exponential growth than in the stationary phase. Cells of the flagellate stage in the life cycle of Antarctic strains contained negligible concentrations of these compounds (Fig. 2D). The absorbance at 323 nm of the media containing cultures of flagellates in log phase of growth was low (≤ 0.0034 cm^{-1}). Cultures of colonial *P. pouchetii* from near Tasmania, the North Sea and the English Channel, grown under identical conditions, in exponential growth phase, and having the same morphology, are similar to the East Australian Current material in having substantially less of the 323 nm absorbing material per μg of chlorophyll *a* than the Antarctic strain (Table 1). The reasons for this marked difference in the amount of UV-B absorbing compounds are not clear.

Table 1. *Phaeocystis pouchetii.* Absorbance at 323 nm of 50 °C-extracted 323 nm absorbance per µg chlorophyll *a* of colonial strains from Antarctic (A1-3), Port Esperance, Tasmania (PE.2), East Australian Current (165-7) and English Channel (Veldhuis and 540)

Strain	Absorbance
A1-3	0.1173
PE.2	0.0195
165-7	0.0143
Veldhuis	0.0048
540	0.0047

Table 2. *Phaeocystis pouchetii.* Test of viability using growth rate of colonial cells. Growth rate of unirradiated control was 0.22 doublings per day; concentration of organisms is cells $\times 10^{-3}$ ml^{-1} $\pm$SD

UV-B irradiance (W m^{-2})	Observed live cells at:		Predicted viable cells at Day 0
	Day 0	Day 13	
0	17 ± 6.7	111 ± 62.3	–
0.286	51 ± 25.2	99 ± 28.1	14
0.598	29 ± 7.8	184 ± 109.2	26
1.310	14 ± 13.7	78 ± 34.3	11
2.059	0.3 ± 0.5	3 ± 2.76	0.5

Determination of both the proportion of undamaged cells following exposure to enhanced UV irradiation and their viability indicated that the UV-absorbing compounds provide substantial protection to colonial *Phaeocystis pouchetii* cells. Viability studies showed that the live cell concentration, predicted from the growth rate of the unirradiated control, was well within the standard deviation of the observed cell concentration (Table 2). The exception, at an irradiance of 0.28 W m^{-2}, was probably due to the counting procedure. This was the only culture in which the observed growth rate was negative between Days 0 and 2, by which time the predicted value fell within the standard deviation of the observed cell concentration. Thus, the characterization of chlorotic and greatly vesicularized cells as "dead" in the survival studies shown in Fig. 3 provided valid critera by which to quantitate the viable cells in culture after UV-B irradiation.

The efficacy of the UV-absorbing compounds in protecting colonial *Phaeocystis pouchetii* cells from UV radiation is apparent from Fig. 3. The percentage of undamaged (live) cells in cultures of colonial East Aus-

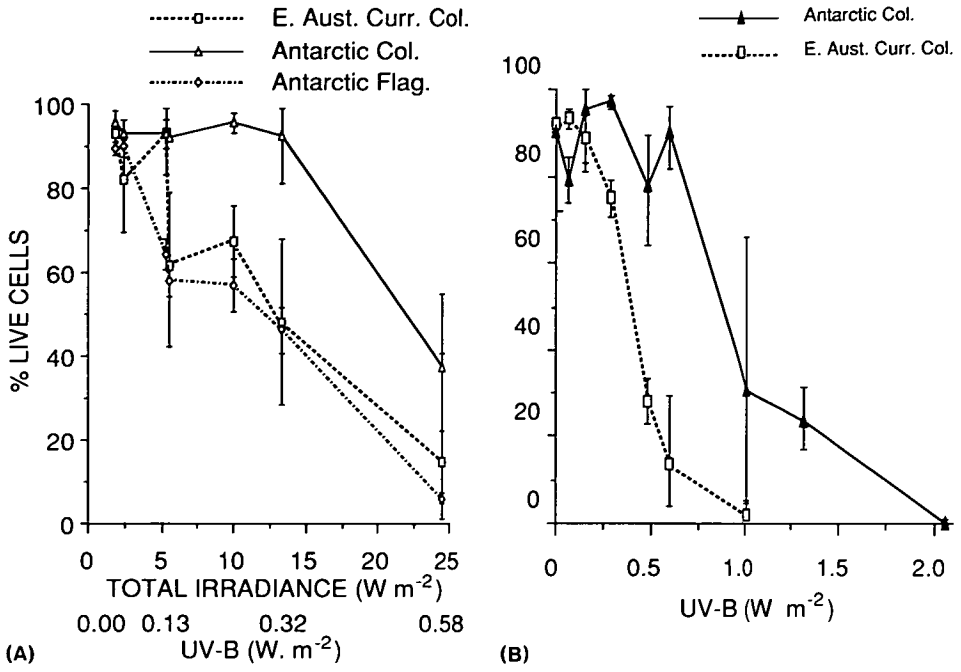


Fig. 3. *Phaeocystis pouchetii.* Percentage of live cells (A) in East Australian Current Colonial (E. Aust. Curr. Col.), Antarctic colonial (Antarctic Col.) and Antarctic flagellate (Antarctic Flag.) cultures exposed to increasing light intensities provided by Suntest UV/white light source; and (B) in Antarctic and East Australian Current colonial cultures irradiated with various intensities of UV-B and constant photosynthetically active radiation (PAR) and UV-A. Error bars represent standard errors calculated from Zar (1984)

tralian Current and Antarctic motile cells decreased markedly with increasing total irradiance (Fig. 3A). In contrast, Antarctic colonial cells were apparently unaffected when the total irradiance was lower than 13.27 $W m^{-2}$, corresponding to an experimental UV-B irradiance of 0.32 $W m^{-2}$. This irradiance is approximately 60% of peak and 150% of average surface irradiance measured at the Australian Antarctic station of Casey at Latitude 67°S (Wood personal communication). At higher irradiances, mortality of colonial cells increases at about the same rate as motile cells. The percentage of live East Australian Current colonial cells was equivalent at each UV-B irradiance, irrespective of whether this was under increasing total radiation (Fig. 3A) or UV-B alone (Fig. 3B). This contrasted with the Antarctic colonial strain. Under increasing total irradiance the proportion of live cells declined to approx 40% when exposed to a total irradiance of 24.5 $W m^{-2}$, of which 5.81×10^{-1} $W m^{-2}$ was UV-B (Fig. 3A). Increasing UV-B irradiance alone produced no significant mortality until irradiance exceeded 0.60 $W m^{-2}$ (Fig. 3B), suggesting inhibition by elevated PAR under increasing total irradiance, or sustained greater cell damage since the UV-B spectrum was only attenuated to Distribution 6 (Fig. 1) in all cases. This UV-B irradiance approximates peak Antarctic coastal surface irradiance (Wood personal communication).

Production of UV protecting pigments is enhanced by exposure to UV radiation in higher plants (Caldwell 1981) and the cyanobacterium *Nostoc commune* (Scherer et al. 1988). Sublethal irradiance of Antarctic colonial *Phaeocystis pouchetii* with UV-B was also found to significantly increase the absorbance per cell at 323 nm (Fig. 4). Only three of the experimental irradiances were sublethal to East Australian Current colonial *P. pouchetii*, but these appeared to reduce rather than promote pigment production. Thus, the rate of UV-B-absorbing compound production by the colonial stage of Antarctic *P. pouchetii* is responsive to ambient UV-B climate. Our data show that the resulting increase in UV-B absorbance by the Antarctic strain would enhance their survival. Should the UV-B screening prove insufficient, cells are damaged or die and production rates of UV-B

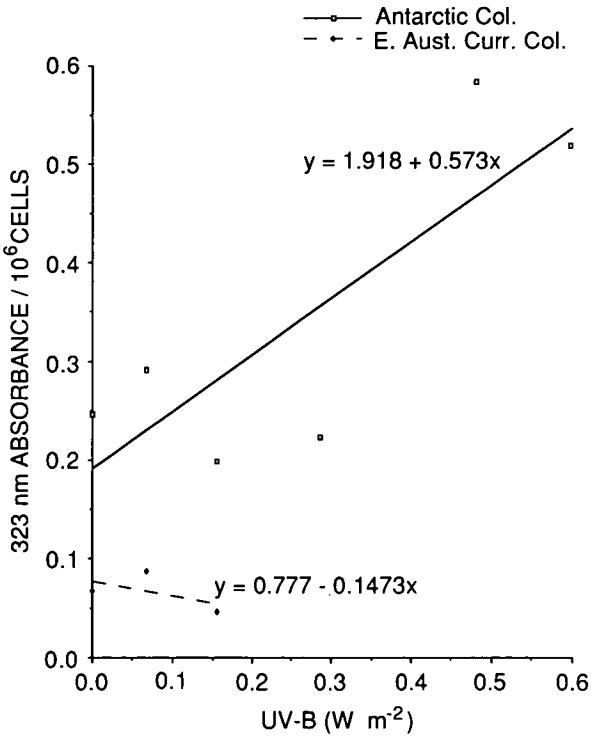


Fig. 4. *Phaeocystis pouchetii.* UV-B absorbing pigment per cell plotted against sublethal UV-B irradiance at constant PAR and UV-A. Regression for Antarctic Colonial (Antarctic Col.) strain was significant ($0.02 < P < 0.05$), while that for the East Australian Current Colonial (E. Aust. Curr. Col.) was not

absorbing compounds would decline, increasing the vulnerability of the remainder of the population to further UV-B damage. However the colonial stage of Antarctic *P. pouchetii* carries a substantial complement of UV-B-absorbing material irrespective of past UV climate (Fig. 2A) which would help mitigate such breakdown of UV-B protection in the wild. In contrast, the East Australian Current colonial strain produces less pigment and is apparently unable to increase the rate of production under UV-B stress.

The absorption of Antarctic colonial *Phaeocystis pouchetii* Strain A1–3 at 323 nm was 1.219 cm^{-1} at a chlorophyll *a* concentration of 810 $\mu g\, l^{-1}$. In the marginal ice-edge zone near the Antarctic coast, the concentration of *P. pouchetii* reached 6.0×10^7 cell l^{-1}, which corresponds to a chlorophyll *a* concentration of 4.59 $\mu g\, l^{-1}$ (Davidson and Marchant 1991). Relating the chlorophyll *a*-normalized 323 nm absorbance in culture to the chlorophyll concentration of the Antarctic *P. pouchetii* bloom indicates that at such a concentration absorbance would be about 80% m^{-1} at 323 nm. Although this predicted value is only approximate (combining data from different laboratory and field studies), it does indicate that *P. pouchetii* is likely to provide UV-B protection for organisms present in the water column at the same time such as diatoms and green algal flagellates that contain little or no UV-B absorbing pigments (Yentsch and Yentsch 1982, Raymond personal communication, Davidson and Marchant unpublished data).

Acknowledgements. We thank S. Wright for discussions, and S. Jeffrey and S. Blackburn for providing algae from the CSIRO Culture Collection of Micro-algae, and acknowledge financial support from the Australian Research Council.

Literature cited

Caldwell, M. M. (1981). Plant responses to solar ultraviolet radiation. In: Lange, O. L., Nobel, P. S., Osmond, C. B., Ziegler, H. (eds.) Encyclopedia of plant physiology. New series. Physiological plant ecology. 1. Springer-Verlag, New York, p. 169–197

Calkins, J., Thordardottir, T. (1980). The ecological significance of solar UV radiation on aquatic organisms. Nature, Lond. 283: 563–566

Carreto, J. I., Carignan, M. O., Daleo, G., De Marco, S. G. (1990). Occurrence of mycosporine-like amino acids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? J. Plankton Res. 12: 909–921

Davidson, A. T., Marchant, H. J. (1987). Binding of manganese by Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release. Mar. Biol. 95: 481–487

Davidson, A. T., Marchant, H. J. (1991). Protist interactions and carbon concentration during a *Phaeocystis*-dominated bloom at an Antarctic coastal site. (in preparation)

Dunlap, W. C., Chalker, B. E., Oliver, J. K. (1986). Bathymetric adaptations of reef-building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. J. exp. mar. Biol. Ecol. 104: 239–248

Dunlap, W. C., Williams, D. McB., Chalker, B. E., Banaszak, A. T. (1989). Biochemical photoadaptation in vision; U.V.-absorbing pigments in fish eye tissues. Comp. Biochem. Physiol. 93B: 601–607

Frederick, J. E., Snell, H. E. (1988). Ultraviolet radiation levels during the antarctic spring. Science, N.Y. 241: 438–440

Fryxell, G. A., Kendrick, G. A. (1988). Austral spring microalgae across the Weddell Sea ice edge; spatial relationships found along a northward transect during AMERIEZ 83. Deep-Sea Res. 35: 1–20

Garrison, D. L., Buck, K. R., Fryxell, G. A. (1987). Algal assemblages in the antarctic pack ice and in ice-edge plankton. J. Phycol. 23: 564–572

Hardy, J., Gucinski, H. (1989). Stratospheric ozone depletion: implications for marine ecosystems. Oceanography, Wash. 2: 18–21

Jacka, T. H. (1983). A computer data base for Antarctic sea ice extent. A.N.A.R.E. Res. Notes 13: 1–54

Jerlov, N. G. (1950). Ultra-violet radiation in the sea. Nature, Lond. 166: 111–112

Loeblich, A. R. III, Smith, V. E. (1968). Chloroplast pigments of the marine dinoflagellate *Gymnodinium resplendens*. Lipids 3: 3–15

Lorenzen, C. J. (1967). Determination of chlorophyll *a* and phaeopigments: spectrophotometric equations. Limnol. Oceanogr. 12: 343–347

Maske, H. (1984). Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake. J. Plankton Res. 6: 351–357

Roberts, L. (1989). Does the ozone hole threaten antarctic life? Science, N.Y. 244: 288–289

Scherer, S., Chen, T. W., Böger, P. (1988). A new UV-A/B protecting pigment in the terrestrial cyanobacterium *Nostoc commune*. Pl. Physiol. 88: 1055–1057

Shibata, K. (1969). Pigments and UV-absorbing substance in corals and a blue-green alga living in the Great Barrier Reef. Pl. Cell Physiol., Kyoto 10: 325–335

Sieburth, J. McN. (1960). Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters. Science, N.Y. 132: 676–677

Sivalingam, P. H., Ikawa, T., Yokohama, Y., Nisizawa, K. (1974). Distribution of 334 UV-absorbing-substances in the algae, with special regard of its possible physiological roles. Botanica mar. 17: 23–29

Smith, R. C., Baker, K. S. (1979). Penetration of UV-B and biologically effective dose-rates in natural waters. Photochem. Photobiol. 29: 311–323

Smith, R. C., Baker, K. S. (1989). Stratospheric ozone, middle ultraviolet radiation and phytoplankton productivity. Oceanography, Wash. 2: 4–10

Smith, W. O., Jr. (1987). Phytoplankton dynamics in marginal ice zones. Oceanogr. mar. Biol. A. Rev. 25: 11–38

Verity, P. G., Villareal, T. A., Smayda, T. J. (1988). Ecological investigations of blooms of colonial *Phaeocystis pouchetii* – I. Abundance, biochemical composition, and metabolic rates. J. Plankton Res. 10: 219–248

Vincent, W. F. (1988). Microbial ecosystems of Antarctica. Cambridge University Press, Cambridge

Voytek, M. A. (1990). Addressing the biological effects of decreased ozone on the Antarctic environment. Ambio 19: 52–61

Wood, W. F. (1989). Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultra-violet radiation. Aquat. Bot. 33: 41–51

Worrest, R. C. (1983). Impact of solar ultraviolet-B radiation (290–320 nm) upon marine microalgae. Physiologia Pl. 58: 428–434

Wright, S. W., Shearer, J. D. (1984). Rapid extraction and high performance liquid chromatography of chlorophylls and carotenoids from marine phytoplankton. J. Chromat. 294: 281–296

Yentsch, C. S., Yentsch, C. M. (1982). The attenuation of light by marine phytoplankton with special reference to the absorption of near-UV radiation. In: Calkins, J. (ed.) The role of solar ultraviolet radiation in marine ecosystems. Plenum, New York, p. 691–706

Zar, J. H. (1984). Biostatistical analysis. 2nd ed. Prentice-Hall, New Jersey

Progress in Phycological Research, Vol. 8 (F.E. Round/D.J. Chapman, eds)
Biopress Ltd., 1992

The biology and ecology of *Phaeocystis* (Prymnesiophyceae)

ANDREW T. DAVIDSON and HARVEY J. MARCHANT

Australian Antarctic Division, Channel Highway, Kingston, Tasmania 7050, Australia

1. INTRODUCTION

Phaeocystis is a marine phytoplanktonic genus in which two species are presently recognised, *P. pouchetii* (Hariot) Lagerheim and *P. scrobiculata* Moestrup. However, Jahnke and Baumann (1986) and Baumann and Jahnke (1986) separate *P. globosa* from *P. pouchetii* on the basis of colony morphology while Fryxell and Kendrick (1988) and Sournia (1988) indicate that the status of the species *P. pouchetii* is uncertain at present. Due to these taxonomic uncertainties we adopt the view of Parke *et al.* (1971) that the species is best referred to as *Phaeocystis pouchetii "sensu lato"* and in that way all references to *P. pouchetii* in this chapter are meant. *P. pouchetii* is apparently the most abundant and widespread species of *Phaeocystis* and the literature relating to this species is extensive. *P. pouchetii* has a world-wide distribution. In high latitudes its blooms can attain very high concentrations of cells which dominate the phytoplankton over large areas (e.g. Savage 1930; Hart 1942; Lancelot 1983; Barnard *et al.* 1984; Chang 1984; Fryxell and Kendrick 1988; Eilertsen 1989; Lutter *et al.* 1989; Davidson and Marchant 1992). Because of its abundance and the diversity of its extracellular products, *P. pouchetii* is one of the most important phytoplanktonic organisms in polar and subpolar seas (Lancelot *et al.* 1987; Davidson and Marchant 1992). So conspicuous are the blooms of *P. pouchetii* that the colloquial names "Tasman Bay slime" (Chang 1983), "baccy juice", "fishermans sign", "weedy water" and "stinking water" (Orton 1923; Savage 1930) have been coined to describe them.

Progressive eutrophication of the Wadden Sea has led to this alga increasing in abundance and duration of its bloom to the extent that it has become a weed (Cadée and Hegeman 1974, 1979, 1986; Verity *et al.* 1988b). Blooms of *P. pouchetii* are avoided by fish, clog commercial fishing nets (e.g. Savage 1930; Chang 1983), are of low nutritional value and appear detrimental to the growth and reproduction of shellfish and metazooplankton (e.g. Walne 1970; Pieters *et al.* 1980). They also contaminate the sea floor and high tide mark with mucilage (Grontved 1960), which becomes anoxic with bacterial activity. This results in avoidance by fish and causes widespread mortality amongst the benthic infauna and littoral invertebrates (Rogers and Lockwood 1990). Sea foam, derived from the dissolved organic carbon released during collapse of *P. pouchetii* blooms, forms massive foam deposits that accumulate on beaches of Northern Europe (e.g. Eberlein *et al.* 1985; Lancelot *et al.* 1987) and smother appendicularians and nematodes (Armonies 1989). Blooms also release prodigious quantities of dimethylsulfide (DMS) which may contribute significantly to acid rain over Scandinavia (Pearce 1988). The economic ramifications of these blooms, coupled with their ecological significance, maintain this species as the focus of considerable research effort.

P. pouchetii is an enigma with respect to its taxonomy, species succession, ecological roles, antibiosis and the fate of its blooms (Fryxell and Kendrick 1988; Verity *et al.* 1988b). To this should be added its life cycle and aspects of its physiology. Thus, despite the obvious impacts of

blooms of this alga (Lancelot *et al.* 1987; Davidson and Marchant 1992) and the wealth of research published about it, knowledge of its relationship with the biotic and abiotic environment remains relatively poor (Weisse *et al.* 1986). Therefore, a review of current knowledge concerning this alga appeared timely and useful.

There are two main stages in its life history, a biflagellate motile stage and colonial form. With few exceptions, blooms of *P. pouchetii* are composed of colonial stage cells. This stage confers intriguing abilities to which *P. pouchetii* must owe much of its prevalence. Transition from the flagellate to the colonial phase results in each cell losing part of its autonomy (Verity *et al.* 1988b). Each colonial cell exudes large quantities of its photoassimilated carbon (e.g. Lancelot 1984a), the majority as mucopolysaccharides, which perform both a structural function by contribution to the colony matrix and a physiological role by acting as a carbon source in dark catabolism (Lancelot and Mathot 1985), a reservoir of phosphate (Veldhuis and Admiraal 1987) and a site of UV-B screening compounds (Marchant *et al.* 1991). Colony size and its low nutritional value result in decreased grazing pressure on this alga while its prolific release of compounds such as DMS (Andreae and Raemdonck 1983; Barnard *et al.* 1984; Gibson *et al.* 1990) and acrylic acid (Sieburth 1960) may deter grazers (Estep *et al.* 1990) and hinder microbial decomposition (Sieburth 1960, 1961; Davidson and Marchant 1987).

2. TAXONOMY

Taxonomic delimitation of *P. pouchetii* remains uncertain (Parke *et al.* 1971; Sournia 1988) as the morphology and arrangement of cells within the colony are characters that are insufficiently stable for use in determining species. Taxonomic problems are exacerbated by the life cycle being polymorphic and the life history incompletely known (Fryxell and Kendrick 1988; Sournia 1988). On the basis of differences in periplast scales and "stars" produced by its motile cells there is little doubt (see Sections 3.1 and 3.2.1) that *P. scrobiculata* (Moestrup 1979) is a separate species. Whether the strains of *P. pouchetii* that have different growth habits in the colonial phase of the life cycle and exhibit different temperature tolerances are separate species or simply different strains remains to be ascertained.

The first description of cells now known as *Phaeocystis* was made by Hariot in Pouchet (1892) as *Tetraspora Poucheti* sp. nov. Later, Lagerheim (1893) created the genus *Phaeocystis* then reclassified this alga as belonging to it (Lagerheim 1896). Nine species of *Phaeocystis* have been formally described but only two are currently regarded as sufficiently morphologically distinct to justify species status, namely *Phaeocystis pouchetii* (Hariot) Lagerheim and *P. scrobiculata* Moestrup. Reviews of the genus (Kornmann 1955; Bourrelly 1957; Kashkin 1963) resulted in *Phaeocystis globosa* Scherffel, *P. sphaeroides* Buttner, *P. amoeboidea* Buttner, *P. giraudyi* (Derb. et Sol.), *P. antarctica* Karsten and *P. brucei*

Mangin being relegated to synonymy with *P. pouchetii s.l.* because the nature and arrangement of cells within the colonies were not considered a criteria to justify their taxonomic separation.

The organism described as *Procytella primordialis* (Haeckel 1890) may well be *P. pouchetii*, but if synonymy were ever established, it is recommended that this name be ignored in favour of *Phaeocystis* as "*nomen conservandum*" (Soumia 1988). The species name *P. poucheti* occasionally appears in the literature (e.g. Kommann 1955; Guillard and Hellebust 1971); however the original *Poucheti* was amended to *pouchetii* to comply with the orthographic conventions of the International Code of Botanical Nomenclature (Voss 1983, cited in Soumia 1988). Reference to *Phaeocystis antarctica* Karsten as a blue green alga (Richardson and Whitaker 1979) is erroneous. Its colonies, described as growing extensively in sea ice off Signy Island, are likely to be of *Phaeocystis pouchetii*. The alga *Corymbellus aureus* Green is a colonial prymnesiophyte of similar cell size and gross morphology to *P. pouchetii* and it is possible that mis-identification may have occurred (Fryxell and Kendrick 1988). The former, however, forms simple clusters of flagellate cells which are not bounded by a mucilaginous sheath (Green 1976).

P. pouchetii is a cosmopolitan organism with occurrences reported from the tropics (Estep *et al.* 1984; Margalef 1978; Al-Hassan *et al.* 1990) to both northern and southern polar waters (e.g. El-Sayed *et al.* 1983; Barnard *et al.* 1984). It has also recently been isolated by Hallegraeff and Blackburn (pers. comm.) from equatorial waters off Palau. *P. pouchetii* reportedly has thermally distinct strains (e.g. Kommann 1955; Kayser 1970; Grimm and Weisse 1985; Weisse *et al.* 1986; Marchant *et al.* 1991) and fills different ecological roles (e.g. Colijn 1983; Palmissano *et al.* 1986; Fryxell and Kendrick 1988).

Phaeocystis globosa has long been a contender both for separate species status and potential taxonomic precedence over *P. pouchetii*. The description of *P. globosa* by Scherffel (1899, 1900) is more detailed than the description of *P. pouchetii* by Hariot in Pouchet (1892). Soumia (1988) considers that Scherffel better describes both a prymnesiophyte and *Phaeocystis* than do Hariot or Lagerheim's (1896) diagnoses. However, despite *P. globosa* being a more complete description of the species, *P. pouchetii* takes taxonomic precedence and the former has fallen into disuse until recently. Studies by Rick and Aletsee (1989), Jahnke and Baumann (1986, 1987) and Baumann and Jahnke (1986) contend that differences in environmental optima and colonial morphology warrant the re-establishment of *P. globosa* as a separate species. The arguments presented for its re-establishment do not, however, contain ultrastructural data on the flagellate stage, upon which the taxonomy of the species of *Phaeocystis* is based.

Numerous studies of the genus *Phaeocystis* have concluded that colony morphology was an insufficient criterion on which to separate *Phaeocystis* species and that the *globosa*-type colony was one of several morphological varieties of *P. pouchetii* (Kommann 1955; Kashkin 1963; Parke and Dixon 1968; Chang 1983). Further, the suggestion by Rick and Aletsee (1989) that the geographic separation of the colony morphotypes in the North Sea with *P. pouchetii* to the north and *P. globosa* to the south was refuted as early as 1930 by Savage who found *pouchetii*-type *Phaeocystis* in the Southern Bight of the North Sea. This led Ostenfeld to rescind his claim made in 1910 (cited in Savage 1930) that the species distributions were mutually exclusive. Analysis of the elemental composition and growth dependence of *pouchetii*-type and *globosa*-type *Phaeocystis* by Jahnke (1989) did little more than demonstrate already documented differences in thermal strain and physiology that result from inhabiting different environments. Consequently, we conclude that *P. globosa* represents one of the morphological forms of *P. pouchetii s.l.* This does not, however, preclude the use of these names as terms descriptive of the *Phaeocystis* morphotype under investigation.

While it is unlikely that *Phaeocystis pouchetii* and *P. globosa* will become separate species, it is possible that *P. pouchetii* may eventually be found to comprise a number of species (Fryxell and Kendrick 1988; Soumia 1988). It will require chemotaxonomy and more detailed life history and ultrastructural studies to ascertain whether or not this is the case. If *P. pouchetii* is found to be a single species it demonstrates truly remarkable morphological, ecological and physiological plasticity. In contrast, the related genus *Chrysochromulina*, which also has a global distribution, contains at least forty seven described species (Estep and MacIntyre 1989).

3. CELL STRUCTURE AND LIFE CYCLE

3.1 *P. scrobiculata*

It is yet to be reported whether *P. scrobiculata* has a colonial stage and, if so, whether it is distinguishable from *P. pouchetii*. The only reports of this species are as preserved flagellates. These differ from *P. pouchetii* in the structure and arrangement of the periplast scales and the arrangement of its thread-like arrays or "stars". The stars of *P. scrobiculata* consist of thread-like material with their proximal ends arranged into a nine-rayed figure (Fig. 1a). The cells are covered by two types of scales. The dimensions of the larger oval scales were reported by Moestrup (1979) as being 0.6×0.45 µm and the small circular-oval scales 0.21 µm in diameter. Hallegraeff (1983) found these larger scales to be 0.41×0.3 µm and the smaller scales 0.1 µm in diameter. The smaller scales, which have a dorsal patternless rim, occurred between, and probably more proximal to the cell than, the larger oval scales (Moestrup 1979). Both scale types bear ridges radiating from a plain centre on their ventral surface and an undecorated dorsal side (Moestrup 1979; Hallegraeff 1983).

3.2 *P. pouchetii*

The life cycle of *Phaeocystis* has only been reported for *P. pouchetii* and this is only partly resolved. It is known to contain at least two different planktonic phases and may also include a benthic stage (Kornmann 1955; Kayser 1970; Verity *et al.* 1988b). Each of these phases is apparently capable of vegetative reproduction. However, differentiation of one phase from another remains largely unknown and surprisingly little ultrastructural work has been reported. In addition, no information is available concerning sexual reproduction of the species or the ploidy of the various cell stages.

3.2.1 *Flagellate cells*

Using isolates from the English Channel, Parke *et al.* (1971) provide the only detailed description of *P. pouchetii* motile cells. These have an anterior depression, two golden brown plastids, the thylakoids of which are arranged in stacks of three with a girdle lamella. The two flagella are of equal length bearing hair points. Arising between the flagella but in a different plane from the flagella bases is a short, stiff, bulbous ended haptonema (Fig. 2a and b). Cells bear a periplast of two layers of organic scales. The scales of the outer layer are almost circular flat plates, 0.18×0.19 µm in size with a perpendicular, outwardly raised rim and usually exhibiting 48 ridges radiating from a rectangular plain centre on both surfaces. The scales of the inner layer are oval, 0.10×0.13 µm in size, with a strongly inflexed rim and 30 ridges radiating from an oval plain centre on both surfaces.

Flagellate cells of *P. pouchetii* produce threads arranged as a five armed "star" array. Each constituent thread is up to 20 µm long, tapering towards its tip. The proximal ends form a pentagon bounded by a membrane (Fig. 1b). These stars remain something of an enigma and few reports shed light on their mode and rate of synthesis or their release from the cells, let alone their function. We have found in shadow cast preparations of Antarctic material that the threads are tubular in section and appear to consist of overlapping segments as well as single pieces (Fig. 1c). The flagellate cells reportedly possess at least two anterior, membrane bound discs which are circular to oval in shape and contain the threads (Parke *et al.* 1971; Pienaar 1991). These discs may either become, or are released into, prominent vesicles which protrude from flagellate cells (Parke *et al.* 1971; Davidson unpubl.) as Davidson (1985) reports that threads were regularly arranged, closely appressed to the inside of the vesicle membrane. Once the vesicle ruptures the arrays are released from the cells giving the characteristic appearance of threads apparently coiled about the cell (Parke *et al.* 1971; Hallegraeff 1983) (Fig. 2b). The arrays presumably then separate and the arms straighten, adopting their characteristic configuration (Figs 1b, 2a).

Cellular structures of *P. pouchetii* including the pentagonal stars, scales and flagella exhibit very little variation over the organism's range (Booth *et al.* 1982; Buck and Garrison 1983; Hallegraeff 1983; Davidson 1985; Marchant and Nash 1986; Lancelot *et al.* 1987; Perrin *et al.* 1987). Reports

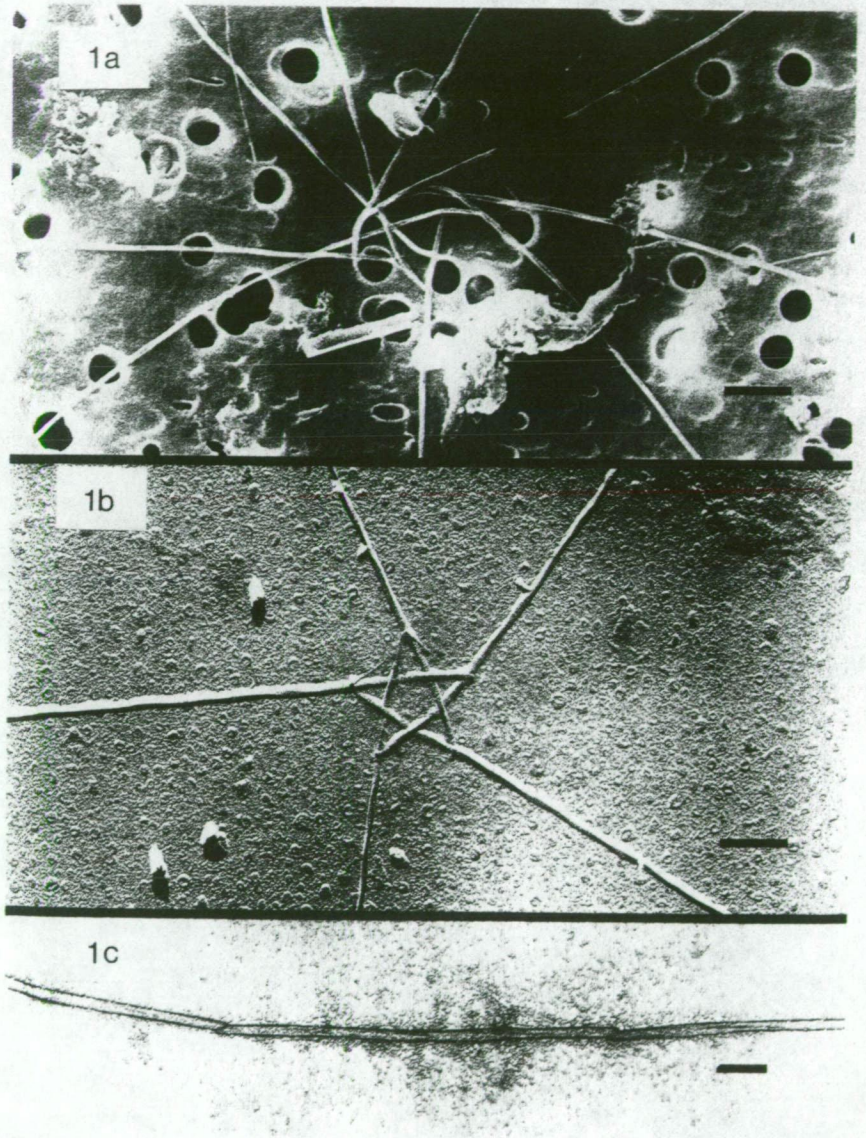


Fig. 1. Electron micrograph of (a) *Phaeocystis scrobiculata* star (micrograph from G. Hallegraeff); (b) *P. pouchetii* star, and (c) detail of the star arms showing the presence of overlapping segments. Scale bars = 2 µm, 500 nm and 200 nm respectively.

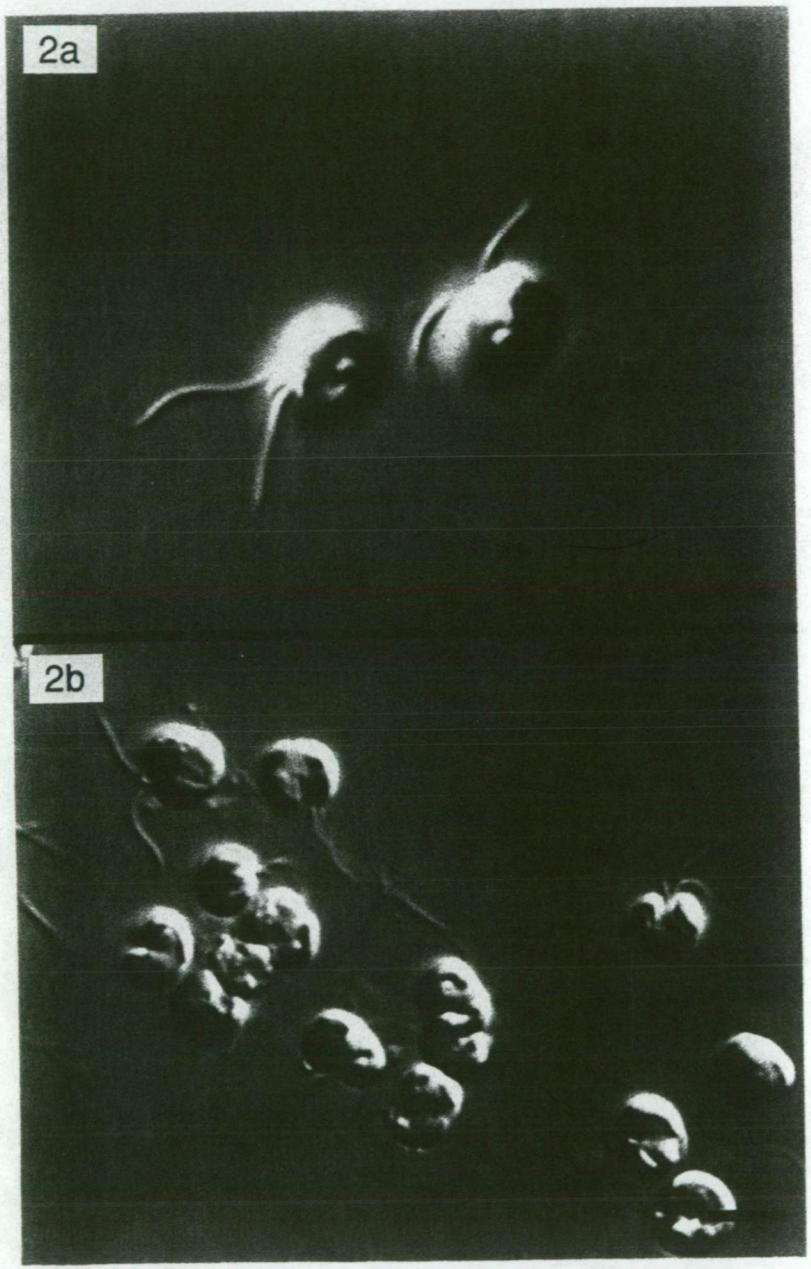


Fig. 2. Nomarski light micrographs of the flagellate stage of *P. pouchetii* showing (a) flagella, haptonema and five-armed stars, and (b) cells surrounded by thread-like star arms (scale bars = 5 µm).

indicate variation in the length of star arms which may reach 50 µm (Buck and Garrison 1983; Lancelot *et al.* 1987) to 100 µm (Fryxell 1989) and circular plate scales which reach 0.25 µm diameter (Hallegraeff 1983) but there is no compelling morphological evidence at this stage to suggest that *Phaeocystis pouchetii* is not a single species.

Kornmann (1955) suggested that the flagellates may be subdivided into three types, all of which are capable of vegetative multiplication, namely:

1. An asexual "swarmer" (between 4.5 to 8 µm diameter)
2. A microzoospore (3 to 5 µm)
3. A macrozoospore

Doubt exists as to the validity of these as serparate stages. The macrozoospore is of unspecified size and uncertain significance in the life cycle. Kornmann (1955) provides the only record of this stage but claims that Scherffel (1899) also described a colony containing macrozoospores. Essentially all recent investigations fail to distinguish between the remaining two flagellate stages of *P. pouchetii* on the basis of size. Flagellates reportedly range in size from 3 to 8 µm (Kornmann 1955; Parke *et al.* 1971; Buck and Garrison 1983; Hallegraeff 1983; Fryxell 1989; Lancelot *et al.* 1987), the same as that reported by Verity *et al.* (1988b) for colonial cells. However, Kornmann (1955) proposed that this range in cell size contains the microzoospore and "swarmer" cell stages which differ from each other in size alone as their function in the life cycle is indistinguishable. It remains unclear whether or not there is more than one motile stage in the life cycle of *P. pouchetii*. The observed variations could be due to environment, phenotypic variability or changing size during the cell cycle.

3.2.2 *Non-motile single cells*

In addition to these flagellate cells, Kornmann (1955) and Kayser (1970) observed a solitary non-motile cell type. Kornmann (1955) described the formation of this stage from flagellate cells which in turn formed colonies. In contrast, Kayser (1970) reported that this stage attaches to solid surfaces and releases new, free single cells and colonies into the water column. These non-motile single cells were proposed as being benthic or attached to motile particles in nature. Whether or not both authors refer to the same cell type is uncertain.

3.2.3 *Colonial cells*

It is in the colonial form ("palmella"-stage) that *Phaeocystis pouchetii* blooms and is most conspicuous. Colonies may reach 2 cm in diameter (Gieskes and Kraay 1975; Verity *et al.* 1988a) and exhibit the physiological peculiarities for which the species is renowned. In spite of the widespread occurrence and ecological importance of the colonial stage, there is only one report of its ultrastructure (Chang 1984). Colonial cells lack body scales, haptonema and flagella and are embedded in a mucilaginous matrix (Fig. 3a, b, c and d). Chang (1984) proposes that chrysolaminarin vesicles, which he

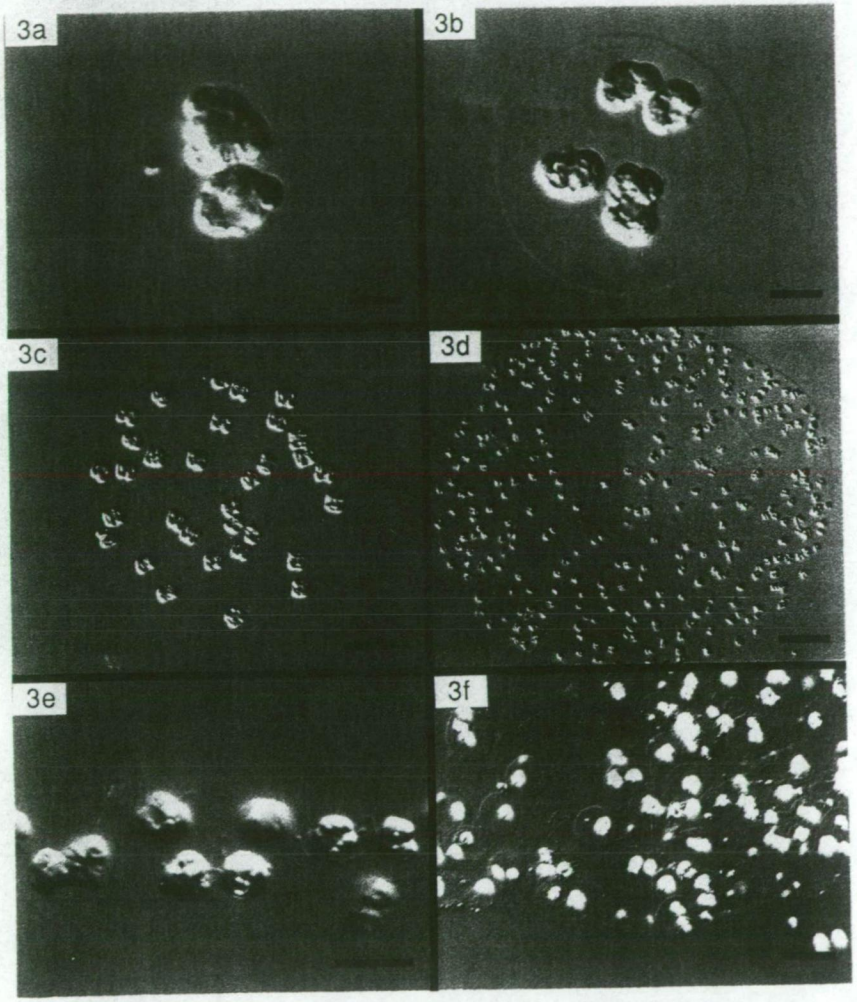


Fig. 3. Nomarski light micrograph of *P. pouchetii* colonies showing (a), (b), (c) and (d) a range of colony diameter sizes (scale bars = 5, 5, 20 and 50 µm respectively), (e) a section of an elongate colony in which cells are differentiating into the flagellate stage (scale bar = 15 µm), and (f) part of a large senescent colony in which small colonies are being formed (scale bar = 30 µm).

observed protruding through the plasmalemma, deposit a multi-layered mucilaginous envelope. Our observations of Antarctic colonial *Phaeocystis* have revealed no indication of multiple mucilaginous layers. Chang (1984) claims that in all other respects this stage is indistinguishable from the flagellate stage.

The ultrastructural variability of the colonial stage over its range remains unknown but gross colony structure and arrangement of cells can be extremely variable. Colonies range in size from approximately 10 µm containing a single cell to 2 cm containing thousands of cells (Gieskes and Kraay 1975; Verity *et al.* 1988a) (Fig. 3a, b, c and d). The shape of colonies can vary greatly. Spherical (Fig. 3a, b, c and d), ovoid, elongate (Fig. 3e), lobed (Kornmann 1955; Bätje and Michaelis 1986), irregular flat discs (Kayser 1970) or dense aggregated mats (Chang 1983; Nichols *et al.* 1991) have been reported. Similarly, the distribution of cells within colonies can be the random (*globosa*-type) (Fig. 3a, b, c and d), clumped (*pouchetii*-type) or aggregated at one pole (Davidson unpubl.). Emigration of cells results in "ghost" colonies, bereft of cells (Verity *et al.* 1988b).

The cells of the colonial stage secrete 5-80% of their photoassimilated carbon (e.g. Guillard and Hellebust 1971; Gieskes and Bennekom 1973; Colijn 1983; Lancelot 1983; Laanbroek *et al.* 1985; Veldhuis *et al.* 1986a), much of which is devoted to matrix formation (Lancelot and Mathot 1987). The rewards for this metabolic expense of colony formation are apparently considerable. The colony creates its own microenvironment in which it can manipulate trace metal concentrations (Lubbers *et al.* 1990), protect against bacterial attack (Davidson and Marchant 1987), store photosynthate for catabolism in the dark (Lancelot and Mathot 1985) and retain UV-B absorbing compounds which shield the cells from damaging wavelengths (Marchant *et al.* 1991). Furthermore, the colony tends to function as a biological entity rather than a passive aggregation of cells (Lancelot and Mathot 1985; Veldhuis and Admiraal 1985; Lancelot *et al.* 1986; Veldhuis *et al.* 1986a). Verity *et al.* (1988a) showed that the colonies had endogenous regulation of formation, growth, senescence (Fig. 3f) but more importantly were capable of cleavage to produce smaller daughter colonies. Such sophisticated organization of the colony is reminiscent of the green algal order Volvocales (Verity *et al.* 1998a).

3.2.4 *Life cycle changes*

Differentiation of cell stage by *P. pouchetii* in culture is not entirely predictable. Flagellate cells may persist indefinitely by vegetative reproduction, can be released by colonies (Fig. 3e) and the "swarmers" may reform colonies (Kornmann 1955; Kayser 1970; Parke *et al.* 1971; Verity *et al.* 1988b; Fryxell 1989) (Fig. 4). However, the regulation of differentiation is poorly understood. Colonial cells that experience a rapid decrease in temperature or chronic nutrient deprivation change to the flagellate cell stage and exit the colony (Verity *et al.* 1988b). Once initiated this process is irreversible. This exodus probably accounts for the high numbers of

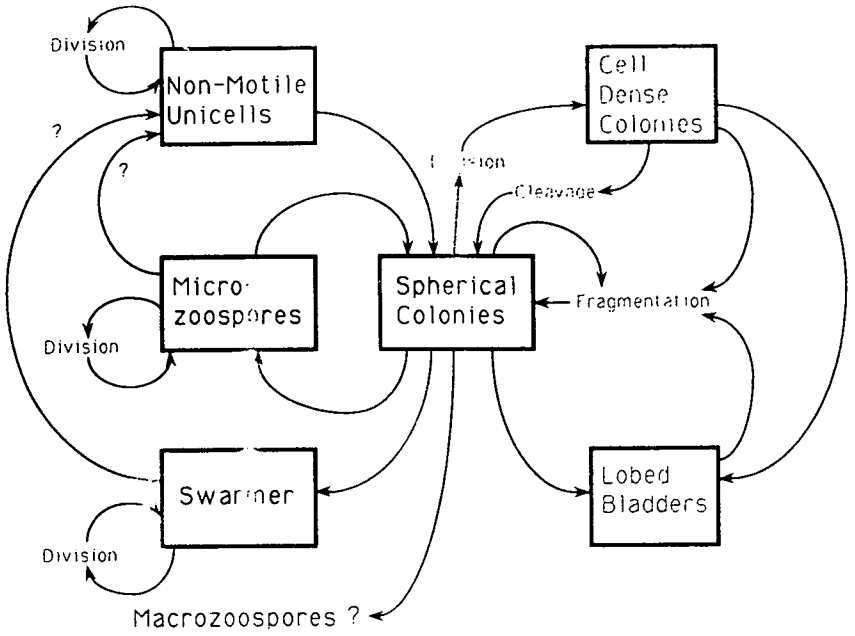


Fig. 4. Current known stages and relationships between stages in the life cycle of *P. pouchetii* (after Veldhuis 1987).

flagellates observed following the decline of colonial blooms (Veldhuis *et al.* 1986b; Davidson and Marchant 1992) and may help explain the apparent sudden disappearance of *P. pouchetii* blooms (Orton 1923; Admiraal and Venekamp 1986). Similar to colonial cultures (see Section 3.1), colonial blooms in the field reportedly release "swarmers" (Jones and Haq 1963) or microzoospores (Veldhuis *et al.* 1986b, Verity *et al.* 1988b). Non-motile unicells may also contribute to the formation of new colonies (Chang 1983). The change from the flagellate to colonial stage may also be stimulated by exudates from diatoms (Boalch 1984). Eutrophication (Guillard and Hellebust 1971) or high phosphate concentration alone (Veldhuis and Admiraal 1987) can also induce the dominance of flagellates in culture. The phosphate induced differentiation of colonial cells to flagellates can be inhibited by simultaneous addition of soil extract (Veldhuis and Admiraal 1987). However, the identity of the constituent/s of soil extract that influence differentiation are unknown.

The over-wintering strategy of *P. pouchetii* is unclear. Only its flagellate stage is commonly observed to persist in the phytoplankton throughout the year (Parke *et al.* 1971; Boalch 1987). Their hardiness led Kornmann (1955), Nøst-Hegseth (1982), Boalch (1984), Veldhuis *et al.* (1986b) and Verity *et al.* (1988b) to propose that the flagellate represents a spore-like stage, a refuge from conditions stressful or lethal to the colonial stage. In

contrast, this stage is capable of rapid vegetative reproduction (Kornmann 1955; Kayser 1970), Verity *et al.* 1988b), occasionally comprising the majority of the *P. pouchetii* bloom (Morris 1971), and by returning to the colonial stage, may contribute to the colonial blooms (Kornmann 1955; Jones and Haq 1963; Cadée and Hegemann 1986; Tande and Båmstedt 1987; Veldhuis and Admiraal 1987; Eilertsen 1989; Fryxell 1989; Davidson and Marchant 1992). Such behaviour better befits description as escape from the confines of colonial metabolism to a proliferation and dispersal phase rather than a "spore" or "refuge". Jones and Haq (1963), Kayser (1970) and Verity *et al.* (1988b) suggest that, alternatively, the flagellate may be part of more complex life cycle changes that result in the development of resistant spores. The attached solitary cell stage has also been proposed as an over-wintering form of *P. pouchetii* (Kayser 1970; Sieburth 1979), a life cycle strategy that would, according to Sieburth (1979), restrict this alga to inshore or coastal waters.

4. DISTRIBUTION AND ABUNDANCE

Phaeocystis scrobiculata has been reported off New Zealand where it was first described (Moestrup 1979), in the East Australian Current (Hallegraeff 1983) and from the equatorial Atlantic (Estep *et al.* 1984) (Fig. 5). The infrequency of reported occurrences of this species may reflect a limited geographic range, low abundance, or problems of distinguishing it from *P. pouchetii*.

Phaeocystis pouchetii is a minor component of the phytoplankton in warm temperate and tropical waters (Fig. 5). Guillard and Hellebust (1971) found that a tropical isolate of this alga from waters off Surinam grew rapidly in culture and noted, in the light of this finding, that the relative absence of *P. pouchetii* in warm neritic waters was unexpected. Al-Hassan *et al.* (1990) subsequently reported a bloom of *P. pouchetii* in Kuwait Bay in the Arabian Gulf, demonstrating that blooms of the species are not excluded from tropical waters. Interestingly, Al-Hassan *et al.* (1990) concluded that such near-surface summer blooms of *P. pouchetii* were probably due to nutrient enrichment by industries and sewage. Thus, in the absence of nutrient enrichment by human activity (Al-Hassan *et al.* 1990) or upwelling (Margalef 1978), oligotrophic tropical waters may not be an environment conducive to the development of its blooms. Observations of the species by Al-Hassan *et al.* (1990), Estep *et al.* (1984), Atkinson *et al.* (1978), Margalef (1978), Hallegraeff (1983), and Hallegraeff and Blackburn (pers. comm.) have extended the range of this species in the tropics beyond that reported in Kashkin (1963).

P. pouchetii is most abundant throughout the cool temperate, sub-polar and polar waters in both the northern and southern hemispheres (e.g. Savage 1930; Lucas 1940; Hart 1942; Jones and Haq 1963; Kashkin 1963; Iverson *et al.* 1979; Eilertsen *et al.* 1981; Bölter and Dawson 1982; Booth *et al.* 1982; Chang 1983; Bodungen *et al.* 1986; Garrison *et al.* 1987; Fryxell and

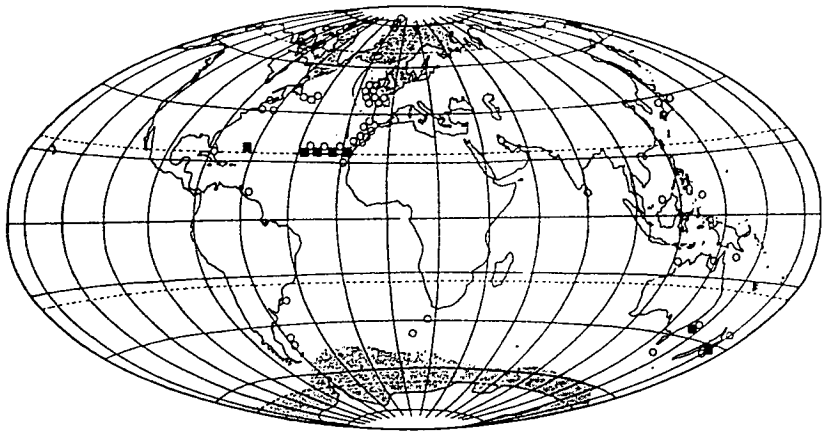


Fig. 5. The global distribution of *P. pouchetii* and *P. scrobiculata*. Regions of frequent *P. pouchetii* occurrence are denoted by stippling, single records of *P. pouchetii* by circles and occurrences of *P. scrobiculata* by squares.

Kendrick 1988; Verity *et al.* 1988a; Holm-Hansen *et al.* 1989) (Fig. 5). Its blooms frequently dominate the phytoplankton (e.g. Joint and Pomeroy 1971; Buck and Garrison 1983) where it is reported by various authors as contributing 40-83% of the total phytoplankton numerical abundance during the productive period (Eilertsen *et al.* 1981; Nøst-Hegseth 1982), in excess of 90% of the total phytoplankton cell number at peak (Joiris *et al.* 1982; Lancelot 1984b; Lancelot *et al.* 1986; Veldhuis *et al.* 1986b), and 65% of the annual primary production (Joiris *et al.* 1982). In polar waters it is often the first species to bloom (e.g. Hart 1942; Lutter *et al.* 1989; Davidson and Marchant 1992) and frequently dominates the algal community within the sea ice (e.g. Fryxell *et al.* 1984; Garrison *et al.* 1987; Garrison and Buck 1989) from where it may seed the water column (Fryxell *et al.* 1984; Fryxell and Kendrick 1988; Garrison and Buck 1989) to dominate the ice-edge blooms (e.g. Buck and Garrison 1983; Garrison *et al.* 1987; SooHoo *et al.* 1987). The flagellate stage of *P. pouchetii* is also a major contributor to the ice edge and open water phytoplankton community during the austral winter (Ashworth *et al.* 1990).

In temperate waters the bloom of *P. pouchetii* occurs after that of diatoms (e.g. Jones and Haq 1963; Gieskes and Kraay 1975; Cadée and Hegeman 1979; Colijn 1983; Cadée 1982; Boalch 1984; Weisse *et al.* 1986; Veldhuis *et al.* 1986b; Lancelot *et al.* 1987). Here it has received much attention from waters of the North Sea where its conspicuous blooms have been known since last century (Pouchet 1892, Scherffel 1899. 1900; Gran 1902; Wulf 1934; Lucas 1940; Künne 1952; Kornmann 1955; Eberlein *et al.* 1985; Owens *et al.* 1989; Riegman *et al.* 1990). These blooms may turn the sea oily, brown and smelly over areas more than 170 km across (Savage 1932) but are usually shortlived, collapsing a few days after their peak

(Rogers and Lockwood 1990). Legendre (1990) proposed that avoidance of *P. pouchetii* by grazers (see Section 5) contributes to the widespread high concentrations of this species.

Continuous plankton records from the North Sea indicate that the abundance of *P. pouchetii* has changed significantly between 1946 and 1987 (Reid 1975; Owens *et al.* 1989). In the northeast Atlantic Ocean and North Sea, the abundance of *P. pouchetii* has declined considerably from their maximum in the late 1940's and autumnal and winter occurrences, which were frequent before the mid 1950's, have largely vanished. Bennekom *et al.* (1975) and Gieskes and Kraay (1975, 1977) also report a decline in *P. pouchetii* abundance between the 1950's and 1970's. Since 1980, though, the occurrence of *P. pouchetii* has apparently increased again. Owens *et al.* (1989) also observed a three yearly cycle in *P. pouchetii* abundance that was due to large scale physical changes which impacted the entire phytoplankton and zooplankton communities. In contrast, *P. pouchetii* abundance in northern European coastal waters, particularly in the region of the Wadden Sea, appears to have increased (e.g. Bennekom *et al.* 1975; Cadée 1986; Cadée and Hegeman 1986; Lancelot *et al.* 1987). It appears that anthropogenic nutrient enrichment of stratified coastal waters off northern Europe (e.g. Foster *et al.* 1983; Lancelot *et al.* 1987) is causing *P. pouchetii* blooms to attain higher numbers and persist longer (Cadée and Hegeman 1986; Lancelot *et al.* 1987; Cadée 1991). In high latitudes, similar to occurrences in enriched waters, *P. pouchetii* attains very high numbers and numerically dominates the phytoplankton for most of the growing season (Eilertsen *et al.* 1981; Davidson and Marchant 1992).

P. pouchetii is most abundant in spring but also occasionally appears in lesser numbers in autumn (Savage 1930, 1932; Jones and Haq 1963; Cadée and Hegeman 1986; Owens *et al.* 1989). In contrast, Eilertsen *et al.* (1981) reported a remarkable year-round occurrence of this species in Balsfjord, spanning a temperature range of 1-7°C while Verity *et al.* (1988a) also reported a winter bloom off Rhode Island. Savage (1930) mentions the occurrence of winter blooms in the North Sea. More recently, however, *P. pouchetii* colonies have reappeared as a feature of the winter phytoplankton in the Wadden Sea (Cadée and Hegeman 1986). This may again reflect the eutrophication of these waters. The seasonal span encompassed by this species again underlines its environmental plasticity.

Blooms of *P. pouchetii* are frequently reported to occur close to the surface (e.g. Margalef 1978; Eilertsen *et al.* 1981; Palmissano and Sullivan 1985; Bodungen *et al.* 1986; Fryxell and Kendrick 1988; Colijn *et al.* 1990) and many of its physiological adaptations point to it being well suited to a high light climate (see Section 6.4). In marked contrast, El-Sayed *et al.* (1983) found *P. pouchetii* from the surface to 150 m depth adjacent to the Ross Ice Shelf, Antarctica. Here 25% of the total primary production in the water column occurred below the depths of 1% light penetration. In addition, Palmissano *et al.* (1986) found this alga could adapt to as little as 16 $\mu Em^{-2}s^{-1}$ light when advected beneath sea ice. Thus, although

characteristically recorded as a species that forms near-surface blooms, *P. pouchetii* is not restricted to these depths.

5. GRAZING

In northern European waters *P. pouchetii* blooms may contribute up to 99% of the total phytoplankton cell number (Lancelot 1984b) and 65% of the annual primary production (Joiris *et al.* 1982). Reports from polar and subpolar waters indicate it plays a similarly important role (e.g. Bölter and Dawson 1982; El-Sayed *et al.* 1983; Bodungen *et al.* 1986; Palmissano *et al.* 1986; Fryxell and Kendrick 1988; Davidson and Marchant 1992). Such a major source of carbon is of considerable significance to higher trophic levels but evidence of the trophic fate of *P. pouchetii* appears contradictory. *P. pouchetii* is variously reported to be of low nutritional value (Sargent *et al.* 1985; Al-Hassan *et al.* 1990; Claustre *et al.* 1990; Nichols *et al.* 1991), incapable of supporting copepod growth and reproduction (Walne 1970; Gabbot and Walker 1971; Verity and Smayda 1989), avoided by invertebrates and fish (Orton 1923; Savage 1930, 1932; Bradstock and MacKenzie 1981; Martens 1981; Chang 1983; Schnack 1983; Ainley *et al.* 1986; Verity and Smayda 1989; Hansen *et al.* 1990; Rogers and Lockwood 1990), inefficiently grazed (Dagg *et al.* 1982; Daro 1985; Verity and Smayda 1989; Hansen *et al.* 1990) or incapable of being grazed (Pieters *et al.* 1980). In contrast, it is also reported that *P. pouchetii* is grazed by protozoa (Admiraal and Venekamp 1986; Weisse and Scheffel-Möser 1990; Davidson and Marchant 1992) and metazooplankton (Lebour 1922; Nicholls 1935; Sieburth 1960; Marr 1962; Jones and Haq 1963; Fretter and Montgomery 1968; Schnack 1983; Weisse 1983; Marchant and Nash 1986; Huntley *et al.* 1987; Tande and Båmstedt 1987; Sargent and Falk-Petersen 1989; Weisse and Scheffel-Möser 1990), that its blooms are not avoided (Jones and Haq 1963; Fryxell *et al.* 1984; Weisse *et al.* 1986) and may provide a substantial proportion of the carbon necessary to support heterotrophic production (Lutter *et al.* 1989).

5.1 *Shellfish*

Feeding studies have shown that few grazers are incapable of ingesting *P. pouchetii*. Pieters *et al.* (1980) reported that the feeding apparatus of *Mytilus edulis* became clogged with colony mucilage and that this probably resulted in starvation. *P. pouchetii* was also found to be a poor food for adult oysters (Gabbot and Walter 1971) and resulted in reduced bivalve spawning success (Walne 1970; Pieters *et al.* 1980). This suggests that the ciliary feeding mode of the bivalves is poorly suited to the ingestion of mucilaginous material. Blooms of this alga did not, however, reportedly cause adult bivalve mortality in British or New Zealand waters (Gabbott and Walker 1971; Pieters *et al.* 1980; Bradstock and MacKenzie 1981) indicating that normal feeding could resume after the *P. pouchetii* bloom receded.

5.2 *Fish*

P. pouchetii blooms, though reportedly not directly toxic to fish (Parke *et al.* 1971), are also avoided by them (Orton 1923; Savage 1930, 1932; Bradstock and MacKenzie 1981; Chang 1983; Rogers and Lockwood 1990). Savage (1932) refers to the slimy and probably unpalatable character of its blooms. Rogers and Lockwood (1990) proposed that the sea floor becomes covered with an anoxic layer of senescent *P. pouchetii*. This was avoided by juvenile flatfish and caused widespread mortality amongst benthic infauna and littoral invertebrates. No other authors have ventured reasons for the observed avoidance.

5.3 *Metazooplankton*

While euphausiids, copepods, cladocerans and meroplanktonic larvae have been shown to graze *P. pouchetii* (Lebour 1922; Nicholls 1935; Jones and Haq 1963; Fretter and Montgomery 1968), the extent of their grazing provides a less coherent picture than those so far discussed. Blooms of this species are often massive but shortlived. The reproductive response time of metazoa, particularly at low temperatures, renders it highly improbable that such grazers as copepods could optimally utilize such a temporally transient substrate (Verity and Smayda 1989). Further, Weisse *et al.* (1986) state that the coincidence of *P. pouchetii* blooms with an absence of copepods observed by Martens (1980, 1981) was not a causal one but instead reflects development of their populations in time. This may also explain the declining copepod numbers observed by Smayda (1973) during a *P. pouchetii* bloom.

A recurrent result of *P. pouchetii* grazing studies is that it is a suboptimal substrate. Biochemical analyses have shown that, in comparison with other phytoplankton, especially diatoms, *P. pouchetii* contains low concentrations of polyunsaturated fatty acids, neutral lipids, essential fatty acids and vitamin C (Sargent *et al.* 1985; Claustre *et al.* 1990; Priscu *et al.* 1990; Nichols *et al.* 1991). This may partly explain reports of poor growth and reproduction in shellfish (Walne 1970; Gabbott and Walker 1971; Pieters *et al.* 1980) and copepods (Rijswijk *et al.* 1989; Verity and Smayda 1989). In addition, the release of organic substances by *P. pouchetii*, particularly DMS (Barnard *et al.* 1984; Gibson *et al.* 1990), acrylic acid (Sieburth 1960; Guillard and Hellebust 1971) and large quantities of carbohydrate (Guillard and Hellebust 1971; Lancelot 1983), may deter organisms from grazing it (Verity and Smayda 1989; Rogers and Lockwood 1990) or occupying the same watermass (Smayda 1973; Martens 1980, 1981). Verity and Smayda (1989), refute this proposition on the basis that if colonies are ingested by some large suspension feeding copepods they cannot be chemically undesirable to these species and may not be to any species. Recently,

however, Estep *et al.* (1990) found in field studies that predation on *P. pouchetii* colonies by three copepod species was dependant upon the physiological state of the alga. *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* avoided actively photosynthetic *P. pouchetii* colonies but ingested senescent ones. Estep *et al.* (1990) also attributed this avoidance to the release of chemical deterrents by actively growing *P. pouchetii*, possibly acrylic acid or DMS. These results are at variance with those of Tande and Båmstedt (1987) and Hansen *et al.* (1990) who found the same copepod species actively grazing freshly cultured *P. pouchetii*.

As discussed earlier (see Section 3.2.1), flagellate *P. pouchetii* are 3 to 8 μm in diameter (Kornmann 1955; Kayser 1970; Parke *et al.* 1971). The nutritional value of the flagellates is equivalent to *Chaetoceros* for *Calanus* spp. (Tande and Båmstedt 1987). Nichols *et al.* (1991) showed that the flagellate had a higher lipid and fatty acid content than the colonial stage, making it a better source of nutrition. The size of the colonial stage ranges from little larger than the flagellate to 2 cm in diameter. Thus alternation by *P. pouchetii* from the colonial to flagellate stage allows changes in size of over three orders of magnitude (Parke *et al.* 1971; Gieskes and Kraay 1975; Verity *et al.* 1988a). This would have profound effects on the capacity of zooplankton to graze its blooms and the efficiency with which they are grazed, potentially making this alga relatively unavailable to metazooplankton (Reynolds *et al.* 1982; Verity *et al.* 1988b). Schnack (1983) and Miller and Hampton (1989) reported that omnivorous copepods and those with a "mixed and raptorial feeding mode" grazed *P. pouchetii* in Antarctic waters while filter feeders did not. In spite of the ability of krill to graze this alga, it appears that diatoms make up most of the diet of krill in Antarctic waters (Meyer and El-Sayed 1983; Miller and Hampton 1989). Raptorial grazing by copepods on *P. pouchetii* was reported by Weisse (1983) who found that colonies between 50 and 350 μm diameter were preferred. *Calanus finmarchicus* grazed *P. pouchetii* colonies from 30 to 100 μm diameter at the same rate as diatoms and tended to ignore large colonies (Hansen *et al.* 1990). They also found that animals at later developmental stages preferred the larger food particles. Large suspension feeding copepods also appear capable of grazing *P. pouchetii* (Huntley *et al.* 1987; Tande and Båmstedt 1987) while Miller and Hampton (1989) suggest that this alga is avoided by most small metazooplankton.

Calanus finmarchicus and *C. hyperboreus* can both grow on a diet of *P. pouchetii* (Tande and Båmstedt 1987) as can *Acartia clausi* and *Temora longicornis* (Weisse 1983). However, Verity and Smayda (1989) found that grazing rates of *Acartia hudsonica* and *A. tonsa* on *P. pouchetii* flagellate cells and colonies were very low and that egg production was related to the concentration of diatoms alone. When offered only *P. pouchetii*, egg production of the copepods fell to the level of the starved controls. In a field study Claustre *et al.* (1990) found that, although *P. pouchetii* comprised 97% of the algal biomass, the diatoms comprised some 74% of the copepod diet.

Zooplankton are limited in the extent and rate of their grazing by feeding behaviour and the structure and geometry of their feeding apparatus. These change with the developmental stage of a grazer and determine significantly the particle size available to it. This structural limitation of particle size available to a grazer is of particular importance when considering *P. pouchetii* because of the enormous potential range in size of this alga. Particle size may also be of greater importance than the gelatinous nature of the colonies and the potential unpalatability of this exudate in determining copepod grazing rate on *P. pouchetii* (Schnack 1983) but clogging of feeding appendages has been cited as a major limiting factor (Martens 1981; Schnack 1983).

5.4 *Microheterotrophs*

Microheterotrophs appear capable of grazing *P. pouchetii* (Hollowday 1949; Fryxell *et al.* 1984; Admiraal and Venekamp 1986; Lutter *et al.* 1989; Wassmann *et al.* 1990; Weisse and Scheffel-Möser 1990; Davidson and Marchant 1992). Ciliates, heterotrophic dinoflagellates and choanoflagellates are well suited to rapid population increase in response to the spectacularly rapid development of *P. pouchetii* blooms and Admiraal and Venekamp (1986) suggested that tintinnid grazing was sufficient to limit the duration of its bloom. Although rate of grazing on *P. pouchetii* colonies themselves may be low, "the microbial loop" may form an important link with higher trophic levels (Davidson and Marchant 1992).

5.5 *The fate of* P. pouchetii *blooms*

Selective avoidance of *P. pouchetii* by grazers may contribute to the development of its blooms (Verity and Smayda 1989); indeed, exceptional blooms often involve species such as *P. pouchetii* and *Gyrodinium aureolum* which are avoided by grazers (Holligan 1987; Paerl 1988). Claustre *et al.* (1990) found only 1.5% of the biomass of a *P. pouchetii* bloom was grazed by copepods, the remainder apparently being lost to the pelagic food web. The unique physiology of *P. pouchetii* and growth of this alga relatively unconstrained by grazing mortality results in massive accumulations of organic carbon and nitrogen (Bölter and Dawson 1982; Davidson and Marchant 1992) which sediment within the euphotic zone to give the highest POC and PON sedimentation rates ever recorded (Wassmann *et al.* 1990). However, its blooms apparently contribute little to direct carbon flux to the deep ocean as much of the carbon fixed by this alga is respired by microheterotrophs and bacteria in the upper 100 m (Wassmann *et al.* 1990; Davidson and Marchant 1992). Avoidance of *P. pouchetii* by grazing zooplankton would mean that this alga also contributes little to vertical carbon flux as faeces and moulted exoskeletons (Marchant and Davidson 1991). Thus, despite accounting for a significant proportion of the primary production in the higher latitudes, this alga apparently does little to ameliorate the accumulation of atmospheric CO_2.

6. PHYSIOLOGY

Phaeocystis pouchetii possesses a peculiar physiology (Lancelot *et al.* 1987) and it is in the colonial stage that these peculiarities are exhibited, enabling it to outcompete other algae. Despite the importance and ubiquity of *P. pouchetii*, its physiology, growth and metabolism are not well understood.

6.1 *Growth*

The factors that determine the growth rate of *P. pouchetii* are the subject of some conjecture. It has been found to depend on inorganic nutrient availability, irradiance, and/or temperature (Bätje and Michaelis 1986; Weisse *et al.* 1986; Lancelot and Mathot 1987; Verity *et al.* 1988a). Lancelot and Mathot (1987) found that photosynthetic rate, mucilage production, and thus to some extent increase in colony diameter, are independent of both ambient nutrient concentration and temperature. However, Verity *et al.* (1988a) indicated that irradiance and nitrate concentration appear to be the two major determinants of growth and photosynthetic rates in *P. pouchetii*.

In the only report describing the genesis of a *P. pouchetii* bloom, Bätje and Michaelis (1986) found isolated patches of red-brown discolouration which increase in diameter and spread over the entire area. Thus, both the cells necessary as a seed source for the bloom and the conditions conducive to their proliferation occur simultaneously and independently over a considerable area. This gives rise to patches of *P. pouchetii* which coalesce during development of the bloom. Its outbursts have been attributed to various physical and biotic factors (see Sections 6.3, 6.4); however, the factor/s initiating blooms are unclear (Cadée and Hegemann 1986; Weisse *et al.* 1986; Lancelot *et al.* 1987).

Weisse *et al.* (1986) reported that *P. pouchetii* blooms occur between the spring diatom bloom and the development of the summer phytoplankton assemblage in temperate latitudes. They concluded that *P. pouchetii* does not replace other species but instead provides extra production to the system. This is not true of polar waters where *Phaeocystis* commonly precedes the diatom bloom (Gran 1929, 1930; Smayda 1980; Davidson and Marchant 1992) and seems only partially sustainable in temperate latitudes where *Phaeocystis* is commonly observed to have an antagonistic effect on co-occurring phytoplankton. Proliferation of *P. pouchetii* inhibits the development of populations of other phytoplankton groups (Lucas 1940; Jones and Haq 1963; Smayda 1973; Barnard *et al.* 1984; Admiraal and Venekamp 1986; Bätje and Michaelis 1986; Veldhuis *et al.* 1986b; Weisse *et al.* 1986; Davidson and Marchant 1992). Thus, it is equally possible that the bloom of *P. pouchetii* interrupts or terminates the diatom bloom. Possible reasons for this limitation of other algae include preferential grazing by copepods on diatoms (e.g. Verity and Smayda 1989; Claustre *et al.* 1990), the ability of *P. pouchetii* to outcompete other phytoplankton for

macronutrients (Laandbrock *et al.* 1985), silicate depletion by diatoms (Verity *et al.* 1988a) and accumulation of trace metals by *P. pouchetii* (Davidson and Marchant 1987; Lubbers *et al.* 1990). These are discussed in more detail in Section 6.3.

6.2 *Temperature*

Phaeocystis pouchetii grows in a wide variety of environments. Kashkin (1963) characterized this species as eurythermal and reports indicate that *P. pouchetii* isolated from different thermal environments have different temperature tolerances (Table 1). Growth rates presented are merely indicative as they are not maximum growth rates; these also vary between strains. Thus, *P. pouchetii* has developed many thermally distinct strains. It is thus able to occupy marine environments ranging from polar waters at -2°C (e.g. Davidson and Marchant 1992) to equatorial water with temperatures in excess of 36°C (Al-Hassan *et al.* 1990). No absolute temperature can be proposed for bloom initiation throughout its range (Jones and Haq 1963; Weisse *et al.* 1986). It also appears that no one temperature initiates *P. pouchetii* blooms at a single site (Cadée and Hegeman 1986; Weisse *et al.* 1986). Strains of differing thermal tolerance would be expected to respond differently to temperature change. It may be the rate or magnitude of the change in temperature that elicits a physiological response from the alga (Verity *et al.* 1988a).

Table 1. The temperature tolerance and growth rates of different strains of *P. pouchetii* isolated from tropical, temperate and polar waters.

Temperature at which growth rate calculated (°C)	Temperature range of growth (°C)	Growth rate (Doublings/Day)	Author
2-6	2-12	0.16-0.8	Verity *et al.* 1988a
3-6	Not given	0.4-1.1	Nøst-Hegseth 1982
4	Not given	0.22	Marchant *et al.* 1991
6	≤4-13	0.8-1.3	Guillard and Hellebust 1971
15	5-≥18	Not given	Kayser 1970
15	7-20	3.4	Grimm and Weisse 1985
20	17≥27	1.5-2.0	Guillard and Hellebust 1971

6.3 *Nutrients*

The concentrations of nitrate (Bougard 1979; Eilertsen and Taasen 1984; Bätje and Michaelis 1986; Reigman *et al.* 1990) or phosphate (Jones and

Haq 1963; Bennekom *et al.* 1975; Gieskes and Kraay 1975; Veldhuis *et al.* 1986b) have been suggested as significant determinants of the timing, extent and duration of the *P. pouchetii* bloom. This alga is reportedly tolerant of low concentrations of phosphate (Weisse *et al.* 1986) which may be due to its ability to store phosphate (Veldhuis and Admiraal 1987) and nitrogen (Verity *et al.* 1988a) in the colonial stage. Dissimilar to the flagellate stage, uptake of phosphate by colonial cells is maintained in the dark and the energy requirements for this dark assimilation are met by catabolism of intracolonial carbon (see Section 6.5) (Veldhuis *et al.* 1991). *P. pouchetii* also appears adept at growing under conditions of low nitrate concentration (Verity *et al.* 1988a). While providing both a store of nutrient and carbon substrate, the mucilaginous envelope of the colony does not greatly impede nutrient uptake rates (Veldhuis *et al.* 1987; Veldhuis *et al.* 1991). Jahnke (1989) contends that *P. pouchetii* has a relatively poor capacity to store phosphate and indicates that it would not provide a competitive advantage over diatoms. However, the nutrient uptake dynamics reported by Veldhuis *et al.* (1991) may provide an advantage over organisms limited to nutrient assimilation during exposure to light. In contrast, the proposed tolerance of low macronutrient concentration does not explain the almost exclusive occurrence of flagellate *P. pouchetii* in oligotrophic waters (Estep *et al.* 1984) and nutrient enrichment resulting in the appearance of the colonial stage in tropical waters (Al-Hassan *et al.* 1990).

The observed capacity of *P. pouchetii* to thrive at low macronutrient concentration (Jones and Haq 1963; Bougard 1979; Eilertsen and Taasen 1984) appears advantageous to the species in temperate latitudes where its blooms occur after the diatom bloom (e.g. Eberlein *et al.* 1985; Bätje and Michaelis 1986; Veldhuis *et al.* 1986b; Weisse *et al.* 1986; Veldhuis *et al.* 1988). Apparently, nitrate in particular remains in the water column following the diatom bloom (Bätje and Michaelis 1986; Weisse *et al.* 1986) and this nutrient may well determine the magnitude of the *P. pouchetii* bloom (Bougard 1979; Eilertsen and Taasen 1984; Lancelot *et al.* 1986; Verity *et al.* 1988a; Colijn *et al.* 1990). Data from El-Sayed *et al.* (1983) indicate that *P. pouchetii* blooms result in significantly greater depletion of nitrate than other phytoplankton species. Most authors (Jones and Haq 1963; Bennekom *et al.* 1975; Gieskes and Kraay 1975; Cadée and Hegeman 1986; Weisse *et al.* 1986; Veldhuis *et al.* 1986b; Veldhuis 1987) agree that phosphate depletion eventually limits the *P. pouchetii* bloom, others (Lancelot 1983, 1984b; Lancelot and Billen 1984; Lancelot and Mathot 1985), however, reported nitrogen as being the limiting nutrient.

In contrast to diatoms, *P. pouchetii* has no nutritional requirement for silicate (e.g. Codispoti *et al.* 1990; Stefánsson and Ólafsson 1990). This may account for the appearance of *P. pouchetii* blooms after diatoms in the temperate latitudes. Diatoms exhaust the available silicate during their blooms and several authors (Jones and Haq 1963; Jones and Spencer 1970; Bennekom *et al.* 1975; Gieskes and Kraay 1975; Cadée and Hegeman 1979, 1986; Colijn 1983; Boalch 1984; Veldhuis *et al.* 1986b; Weisse *et al.* 1986; Veldhuis and Admiraal 1987; Verity *et al.* 1988a) propose that this allows

P. pouchetii to utilize the remaining macronutrients. Verity *et al.* (1988a) also showed that in mixed phytoplankton communities, replenishment of silicate results in co-occurrence of *P. pouchetii* and diatoms. In contrast, Laanbroek *et al.* (1985) reported that silicate remains in the water column during the *P. pouchetii* bloom. The decline in silicate after the collapse of the bloom was considered evidence that *P. pouchetii* outcompetes diatoms for nutrients.

No mechanism has been proposed to determine the position of *Phaeocystis pouchetii* in the phytoplankton species succession in polar waters. Limitation of diatom abundance by silicate depletion does not apply to the observed phytoplankton sequence as here *P. pouchetii* is the first species to bloom (see Section 4). It remains to be seen what causes this fundamental difference in its behaviour in polar waters.

The lack of dependence by *P. pouchetii* on silicate concentration may be important in determining its proliferation in coastal waters of the North Sea. Agricultural runoff and domestic waste have enriched these waters with nitrate and phosphate but not silicate. This selectively advantages flagellates at the expense of diatoms (Owens *et al.* 1989) and at least some strains of *P. pouchetii* appear well suited to proliferating in these eutrophic conditions (Lancelot *et al.* 1987). Nutrient enrichment increases the concentration and duration of blooms of this alga (e.g. Lancelot and Billen 1984; Cadée and Hegeman 1986; Lancelot *et al.* 1987). Thus, *P. pouchetii* can proliferate in a broad spectrum of nutrient environments from diatom depleted concentrations to the eutrophic levels encountered in Northern European coastal waters (Veldhuis *et al.* 1987).

Nutrient stress has been observed in this species. At low concentrations it causes increased carbon to chlorophyll *a*, nitrogen and ATP ratios and, under conditions of chronic deprivation, results in the alga assuming the flagellate form (Verity *et al.* 1988b). Low phosphate concentrations also lead to increased carbon excretion rates (Veldhuis *et al.* 1986a), an increase in alkaline phosphatase activity (Admiraal and Veldhuis 1987; Veldhuis and Admiraal 1987) and usage of enzymatically hydrolysable phosphorus (Veldhuis *et al.* 1987). Low nitrate concentration leads to decreased protein synthesis, increased production of polysaccharides (Lancelot *et al.* 1986), and a greater proportion of production being expended on secretion of mucilage than metabolism (Lancelot 1983; Lancelot and Mathot 1987). Such metabolic effects of nutrient deprivation may exclude *P. pouchetii* from blooming in oligotrophic tropical waters.

Highly nutrient enriched culture media have also been reported as increasing the proportion of flagellates in cultured *P. pouchetii* (Kayser 1970; Veldhuis and Admiraal 1985, 1987) and data from Bätje and Michaelis (1986) and Cadée and Hegeman (1986) suggest that the number of flagellate blooms in eutrophic waters of the coastal northern Europe has increased (Owens *et al.* 1989). Colonial and flagellate cells have been observed at all nutrient concentrations that would support cell growth (Veldhuis and Admiraal 1987; Verity *et al.* 1988a). Thus, unlike temperature changes (Verity *et al.* 1988b), change in nutrient concentration

did not elicit a change in cell stage by the entire *P. pouchetii* population. Instead, it caused a shift in the ratio of flagellate to colonial cells (Kayser 1970; Veldhuis and Admiraal 1985). Verity *et al.* (1988b) proposed that such changes may also be determined by endogenous factors such as the possible development of sexuality, or behavioural factors including escape from conditions that are stressful to the colonial stage.

P. pouchetii has been shown to accumulate trace metals (Morris 1971), particularly manganese (Davidson and Marchant 1987; Lubbers *et al.* 1990). This accumulation of up to 75% of the available soluble manganese is caused by photosynthetic CO_2 uptake and O_2 evolution which increases the pH and Eh within the microenvironment of the colony (Lubbers *et al.* 1990). The increased pH resuilts in oxidation of the manganese to an insoluble brown precipitate and Davidson and Marchant (1987) reported that it is the presence of these oxides in the colony matrix that accounts for the characteristic colouration of *P. pouchetii* blooms (e.g. Savage 1930; El-Sayed *et al.* 1983). Colonial *P. pouchetii* also has a demonstrated bacteriocidal capacity (Sieburth 1960; Davidson and Marchant 1987) which would result in limited remineralization of the accumulated manganese. Thus, by accumulating this micronutrient which is essential to plant growth (O'Kelly 1974) and limiting its re-release this alga may mediate the phytoplankton species succession (Davidson and Marchant 1987; Lubbers *et al.* 1990).

Mixotrophy has been proposed for this alga (Chu 1946; Kommann 1955; Jones and Haq 1963; Foster *et al.* 1983; Weisse *et al.* 1986) but not demonstrated. Weisse *et al.* (1986) suggested utilization of diatom ectocrines by *P. pouchetii* while Boalch (1984) found that formation of colonies from the flagellate stage of *P. pouchetii* was enhanced by chemical products of *Chaetoceros*. Weisse *et al.* (1986) also postulated the converse, namely that ectocrines from blooms preceding *P. pouchetii* were inhibitory to its development. No empirical data have been provided to support such an interaction and these suggestions perhaps better reflect the lack of understanding of *P. pouchetii* bloom initiation than any real mixotrophic activity. Addition of soil extract to cultures reportedly enhances growth of *P. pouchetii* and it has been suggested that this represents mixotrophic utilization of terrigenous compounds (Chu 1946; Kommann 1955; Jones and Haq 1963). In contrast, Kayser (1970) found that *P. pouchetii* grew poorly in culture media containing soil extract. Such differences probably reflect the variability in soil quality. Our observations indicate *P. pouchetii* does grow well in media containing soil extract. However, no mixotrophic nutrient sources are obligatory as the colonial stage was successfully maintained over more than six culture generations in Aquil synthetic seawater (Morel *et al.* 1979) from which vitamins were omitted (Davidson, unpubl.).

6.4 *Light*

Reported responses of *P. pouchetii* to light intensity vary. Verity *et al.* (1988a) claim that it is able to utilize light at higher irradiances than other algae. This is consistent with reports of *P. pouchetii* being commonly observed in surface waters. Again, however, this alga exhibits extraordinary environmental plasticity. It is reported to adapt to light climates ranging from 1600 $\mu Em^{-2}s^{-1}$ (Palmissano and Sullivan 1985) to 16 $\mu Em^{-2}s^{-1}$ (Palmissano *et al.* 1986). Eilertsen (1989) and Joint and Pomroy (1981) report high photosynthetic efficiency of both the colonial and flagellate *P. pouchetii* at low light intensities. Palmissano *et al.* (1986) showed that *P. pouchetii* adapted to the low light conditions beneath sea ice by increasing its photosynthetic efficiency three to four fold per unit chlorophyll *a* and two to three fold per cell. This adaptation reportedtly involved increasing its absorption of blue-green wavelengths (SooHoo *et al.* 1987). While able to adapt to low light intensities, saturating light intensities for *P. pouchetii* are reportedly high (Colijn 1983). Lancelot and Mathot (1987) found that low light adapted *P. pouchetii* suffered no significant light inhibition at high intensities and they proposed that this may be due to attentuation of light by the mucilaginous envelope.

The colonial stage of Antarctic strains of *P. pouchetii* strongly attenuated ecologically significant light wavelengths below 370 nm (Marchant *et al.* 1991) irrespective of past UV-B climate. Production of absorbing compounds could, however, be further enhanced by irradiation with UV-B light. Possession of these compounds by the colonial stage provides substantial protection against damage by UV-B radiation. However, the flagellate stage lacks the pigmentation, and temperate, tropical and northern hemisphere colonial strains of *P. pouchetii* contain substantially less of these absorbing compounds and are correspondingly less able to survive UV-B exposure (Marchant and Davidson 1991).

The ice-edge bloom provides much of the production in the Southern Ocean (Smith and Nelson 1986) which is a major nutrient source to sustain the wealth of life at higher trophic levels. *P. pouchetii* is a significant contributor to these blooms (e.g. SooHoo *et al.* 1987; Garrison *et al.* 1987; Fryxell and Kendrick 1988) and producing UV-B absorbing compounds is consistent with its occupancy of these near surface waters. However, spring-time UV-B irradiances are increasing due to stratospheric ozone depletion (Stolarski *et al.* 1986) and these enhanced UV-B irradiances coincide with the ice-edge bloom. This led Marchant and Davidson (1991) to propose that *P. pouchetii* could become increasingly dominant at the expense of diatoms in these waters. This could profoundly affect the food web function, nutritional status and production of higher trophic levels (Section 5). In addition, *P. pouchetii* appears to contribute little to the direct vertical flux of carbon to deep water and the sea floor (Wasmann *et al.* 1990) and would contribute little to carbon flux as faeces and moulted exoskeletons as it is reportly poorly linked to higher trophic levels (Claustre *et al.* 1990). This

may significantly reduce the capacity of the Southern Ocean to act as a sink for atmospheric CO_2 (Marchant and Davidson 1991).

6.5 *Biochemical composition*

The commonly used technique of filtration to separate particulate from dissolved organic matter and cellular from extracellular material results in loss of colony matrix and contents to the filtrate (Bölter and Dawson 1982; Lancelot 1984b; Veldhuis and Admiraal 1985; Veldhuis *et al.* 1986a; Lancelot and Mathot 1987; Verity and Smayda 1989; Rousseau *et al.* 1990; Davidson and Marchant 1992). Thus, it is difficult to discriminate between *P. pouchetii* cell contents, colony contents and extracolonial excretion (Bölter and Dawson 1982; Eilertsen and Taasen 1984; Lancelot 1984b; Veldhuis and Admiraal 1985; Verity and Smayda 1989).

As discussed earlier (Section 3.2.3), the extent to extracellular release by *P. pouchetii* reportedly ranges from 5-80% of its photoassimilated carbon. In addition, the amount of extracellular release by a population also varies over time (Veldhuis *et al.* 1986a). The majority of the substances released are mucopolysaccharides (Guillard and Hellebust 1971; Lancelot and Mathot 1987). These may comprise around 40% of the primary production and are mainly utilized as colony matrix (Lancelot 1984a; Lancelot and Mathot 1987). That which remains within the colony has been described as "apparent excretion" by Verity *et al.* (1988a), reflecting the developing realization that the colony functions more as a single biological entity than a simple aggregation of cells.

High light intensities reportedly cause inhibition of mucilage secretion, suggesting that it may suppress one of the steps in mucilage production (Lancelot and Mathot 1987). Consequently, exposure to high light may explain why some cultures form colonies with high cell densities and little mucilage (Nichols *et al.* 1991). Jahnke (1989) also implicated temperature in the carbon balance of the cells as cultures exposed to a rise in temperature decreased in carbon content.

Early growth of the *P. pouchetii* bloom exhibits rapid photosynthesis with protein synthesis dominating carbohydrate production. By the peak of the bloom, carbohydrate was the dominant end product of photosynthesis (Veldhuis *et al.* 1986a). Supporting these findings, Hickel (1984) found that the ratio of particulate nitrogen to carbon fell sharply during the *P. pouchetii* bloom. However, Verity *et al.* (1988a) showed that *P. pouchetii*, in spite of its prolific release of photoassimilated carbon, had a carbon to nitrogen ratio similar to that of other non-gelatinous phytoplankton. According to Eberlein *et al.* (1985), breakdown of colonies released large amounts of dissolved organic nitrogen. This is at variance with reports of low nitrogen content of colonies (Hickel 1984) and low nitrogen content of the mucilage (Billen and Fontigny 1987; Lancelot *et al.* 1987). Veldhuis *et al.* (1986a) proposed that change in metabolism toward carbohydrate synthesis may represent the formation of storage products for the colony. Such intracolonial storage of macromolecules and their catabolism during dark periods have also been proposed (Lancelot and Mathot 1985; Veldhuis and Admiraal 1985). Thus, the excretion of macromolecules is more than a means of building the colony matrix, rather it participates in the energy balance of the constituent cells. This extracellular but intracolonial storage of photosynthate for catabolism is a metabolic pathway which is facilitated by possession of a colonial phase and highlights the function of the colony as a biological entity rather than a simple aggregation of cells.

Polyunsaturated fatty acids in *P. pouchetii* vary considerably in composition and quantity (Sargent *et al.* 1985; Al-Hassan *et al.* 1990; Claustre *et al.* 1990; Nichols *et al.* 1991). This variation may reflect differences in strains or species. It may also result from the differing physiological strictures, such as maintaining membrane function with changing temperature and increasing unsaturation of fatty acids at lower temperatures (Al-Hassan *et al.* 1990; Claustre *et al.* 1990). Lipid synthesis accounts for a relatively constant 20% of carbon fixed (Lancelot 1984b), however, as discussed in Section 5.3, *P. pouchetii* is low in neutral lipids, polyunsaturated fatty acid and vitamin C. As a consequence, this alga is characterized as being of low nutritional value to grazers (Claustre *et al.* 1990). Differences in the fatty acid and lipid composition between stages in the life cycle of this alga suggest that the flagellate is marginally more nutritious than the colonial stage (Nichols *et al.* 1991).

Protein may comprise between 20 and 42% of the total carbon fixed (Lancelot 1984b). The rate of synthesis is, however, variable and is dependent on light history (Lancelot *et al.* 1986) and nitrate concentration (Lancelot 1984b). While *P. pouchetii* blooms are associated with high DON concentration (Eberlein *et al.* 1985) they also coincide with the lowest seasonal concentrations of dissolved free amino acids (DFAA) (Laanbroek *et al.* 1985) and low concentrations of ammonia (Eberlein *et al.* 1985; Laanbroek *et al.* 1985). These authors proposed that the low concentrations of the latter could be caused by either antibiosis of *P. pouchetii* blooms resulting in decreased heterotrophic decomposition of the available DFAA or the increased ammonia uptake and regeneration by this alga.

One conceptual feature of research into *P. pouchetii* colonies that has emerged recently is the appreciation of the colony as an integral unit rather than being a collection of autonomously functioning cells (Lancelot and Mathot 1985; Veldhuis and Admiraal 1985; Lancelot *et al.* 1986; Veldhuis *et al.* 1986a; Verity *et al.* 1988a). The same is also true of the colonial *P. pouchetii* biomass and production. Biovolume and colonial carbon biomass (Rousseau *et al.* 1990) rather than cell number appear to provide a far better indication of production by *P. pouchetii* blooms (Lancelot and Mathot 1985; Veldhuis and Admiraal 1985; Veldhuis *et al.* 1986a).

6.6 *Photosynthetic pigments*

The concentration of some minor components of the pigment arrays in strains of *P. pouchetii* from opposite hemispheres reportedly differs. Chlorophyll c_3 is ubiquitous in *P. pouchetii* (Vesk and Jeffrey 1987), but the

major carotenoid concentrations were found to vary between strains. Bjørnland *et al.* (1988) found that the main carotenoid pigments in tropical *P. pouchetii* were fucoxanthin, 19'-hexanoyloxyfucoxanthin and 19'-butanoyloxfucoxanthin. Fucoxanthin dominated in the northern hemisphere strains with trace quantities of 19'-acyloxyfucoxanthins (Claustre *et al.* 1990; Gieskes and Kraay 1986) while in the southern hemisphere the dominance of these pigments was reversed with 19'-hexanoyloxyfucoxanthin comprising the majority (Wright and Jeffrey 1987; Nichols *et al.* 1991). Bjørnland *et al.* (1988) claimed the presence of 19'-acyloxyfucoxanthins distinguished the tropical strain from other *P. pouchetii* strains and proposed that this was evidence of *P. pouchetii* being composed of more than one species. This appears unjustified as these carotenoids do occur in other strains (Gieskes and Kraay 1986; Wright and Jeffrey 1987) and their differing abundance could be construed as changes in biochemistry with environment and/or strain. Furthermore, Bjørnland *et al.* (1988) proposed that the acyloxyfucoxanthins comprised a chemosystematic marker. These pigments also occur in *Emiliania huxleyi*, *Pelagococcus subviridis* (Wright and Jeffrey 1987), *Corymbellus aureus* (Gieskes and Kraay 1986) and some dinoflagellate genera (Marchant and Wright, unpubl.) and, not being confined to *P. pouchetii*, they are not definitive of the species. However, *P. pouchetii* has an antagonistic effect on blooms of other algae (Section 6.1) and other acyloxyfucoxanthin-containing algae are sparse in the Southern Ocean. Thus these pigments may be useful as a marker when combined with microscopic examination.

The chlorophyllase activity also varies between strains of *P. pouchetii*. Percent conversion of chlorophyll *a* to chlorophylide *a* was five times greater in a Southern Ocean strain than one isolated from the East Australian Current (Jeffrey and Hallegraeff 1987). The reported differences in pigment complement and chlorophyllase activity may be due to genetic differences at the population or species level but may equally be due to physiological differences imposed by the ambient environment.

6.7 *Extracolonial release*

P. pouchetii blooms are often associated with very high DOC concentrations (e.g. Bölter and Dawson 1982; Eberlein *et al.* 1985; Davidson and Marchant 1992). Extracolonial release of carbon has been referred to as the only "true excretion" by *P. pouchetii* (Verity *et al.* 1988a). Carbon released in this way constitutes around 2-14% of the photoassimilated carbon in naturally occurring populations (Laanbroek *et al.* 1985; Veldhuis *et al.* 1986a; Lancelot and Mathot 1987) and cultures (Veldhuis and Admiraal 1985; Veldhuis *et al.* 1986a). Thus, extracolonial excretion accounts for a relatively small proportion of total production (Cadée 1982). The DOC responsible for the spectacular occurrences of sea foam at the end of *P. pouchetii* blooms in the coastal zone of northern Europe (Eberlein *et al.* 1985; Bätje and Michaelis 1986) appears likely to be derived from collapse and decay of ungrazed production by this alga (Cadée 1982; Verity *et al.* 1988a).

6.8 *Dimethylsulfide production*

A cellular product of marine microorganisms, dimethylsulfoniopropionate (DMSP), which is thought to have an osmoregulatory function but may also be used as a structural component of cells, a buoyancy aid or a bacteriocidal agent (Sieburth 1961, 1964; Barnard *et al.* 1984; Variavamurthy *et al.* 1985), can be cleaved to form acrylic acid and dimethylsulfide (DMS) (Sieburth 1960). DMS and its precursor DMSP are ubiquitous in the biosphere and are a common product of algae (Green 1962; Lovelock *et al.* 1972; Barnard *et al.* 1984; Variavamurthy *et al.* 1985). DMS is released when algae die, are exposed to air or as a consequence of normal metabolism (Barnard *et al.* 1984; Variavamurthy *et al.* 1985). Substantial release of DMS is restricted to a few classes of phytoplankton, mainly belonging to the Dinophyceae and Prymnesiophyceae (Keller *et al.* 1989). Such members of the Prymnesiophyceae as *Hymenomonas carterae* and *Phaeocystis pouchetii* produce three orders of magnitude more DMS per cell than most other groups of phytoplankton (Barnard *et al.* 1984). The abundance of *P. pouchetii* at higher latitudes correlates with greatly elevated concentration of this sulfur compound in the water column (Andrae and Raemdonck 1983; Barnard *et al.* 1984; Pearce 1988), particularly in Antarctic waters (Deprez *et al.* 1986; Gibson *et al.* 1990).

Biogenic DMS production may account for as much as 50% of the natural sulfur emission and 21% of the total global sulfur flux (Andreae and Raemdonck 1983). DMS is rapidly oxidized in the atmosphere to SO_2, methanesulfonate and sulfate (Hatekeyama *et al.* 1985; Yin *et al.* 1986). It is proposed that sulfate particles act as cloud condensation nuclei thereby establishing a mechanism for regulation of global albedo, and thus climate, by marine biological activity (Charlson *et al.* 1987; Bates *et al.* 1987a) (Fig. 6). Additional ecological significance is imparted to these sulfate aerosols as they may contribute 25-50% of the total sulfur acids in Scandinavian air during North Sea phytoplankton blooms (Pearce 1988; Pain 1989).

The world average DMS concentration in sea-water is approximately 2nM (Bates *et al.* 1987b), however, Gibson *et al.* (1990) measured concentrations as high as 290 nM during a near-shore bloom of *P. pouchetii*. Such extraordinarily high concentrations of DMS in the water column led Gibson *et al.* (1990) to speculate that up to 10% of the global DMS flux to the atmosphere may emanate from Antarctic seas. Thus, the rate of DMS evolution by *P. pouchetii*, the dominance in the water column and widespread occurrence of this alga in the higher latitudes make this species a major contributor to the global sulfur budget and possibly able to influence global climate.

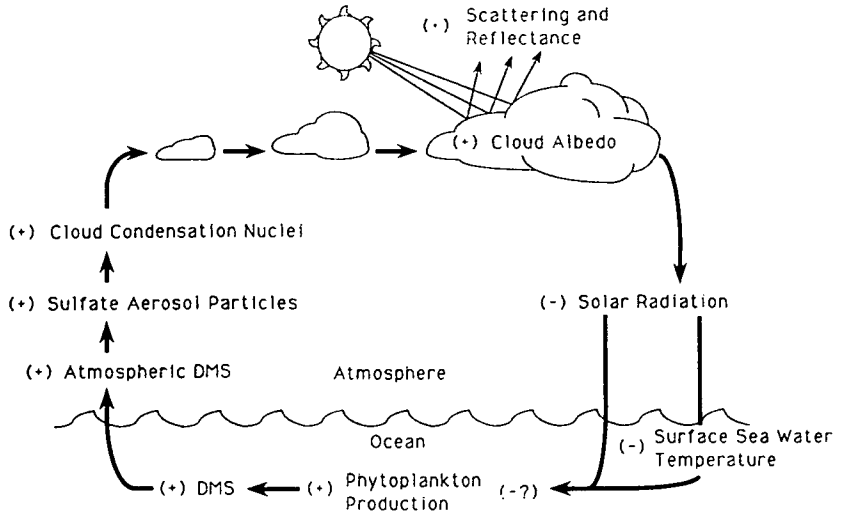


Fig. 6. Schematic diagram of the biogenic sulfur cycle.

6.9 *Acrylic acid and antibiosis*

Photosynthetic products excreted by *P. pouchetii* are readily utilized by bacteria (Hickel 1982; Eberlein *et al.* 1985; Veldhuis *et al.* 1986b; Davidson and Marchant 1987; Verity *et al.* 1988a). By enzymatically cleaving DMSP to produce DMS, *P. pouchetii* also releases equimolar quantities of acrylic acid (Sieburth 1960) and this has been shown to inhibit bacteria (Sieburth 1960, 1961, 1979; Barnard *et al.* 1984). This is supported by the observation that colonies devoid of cells supported bacterial numbers two orders of magnitude greater than those containing cells (Verity *et al.* 1988b). Davidson and Marchant (1987) demonstrated bacterial inhibition in culture and showed that this bacterial defence was confined to the vicinity of each colony rather than throughout the entire culture. Eberlein *et al.* (1985) proposed suppression of bacterial growth by actively growing *P. pouchetii* as the reason for the accumulation of very high concentrations of organic carbon during its blooms and suggested that the proliferation of saprophytic bacteria in sea foam derived from *P. pouchetii* blooms (Eberlein *et al.* 1985; Gunkel 1982) represents loss of the alga's antibiosis upon the collapse of the bloom. Billen and Fontigny (1987) and Lancelot *et al.* (1987) consider that mucilage is deficient in nitrogen and suggest that this would slow its bacterial degradation in comparison with other cells. This supports observations by Rousseau *et al.* (1990) that mucilage synthesis is enhanced at low nutrient concentrations but, as Hickel (1984) points out, it is refuted by the observed proliferation of saprophytic bacteria in sea foam produced by *P. pouchetii*. Inhibition of bacteria would also result in the reduced decomposition of dissolved free amino acids and the low ammonia

concentrations observed by Eberlein *et al.* (1985). Davidson and Marchant (1992) propose a mechanism combining bacterial suppression by acrylic acid with microheterotrophic grazing of bacteria as the reason for the low bacterial concentration during an Antarctic coastal *P. pouchetii* bloom. Bacteria are commonly reported to proliferate following the collapse of *P. pouchetii* blooms (Eberlein *et al.* 1985; Laanbroek *et al.* 1985; Veldhuis *et al.* 1986b; Davidson and Marchant 1987, 1992; Verity *et al.* 1988a). Thus, it appears likely that colonial *P. pouchetii* protects its energetic investment in the colony matrix against bacterial attack by producing acrylic acid and such protection allows the alga to benefit from the various attributes associated with this cell stage. If this is the case, as proposed by Barnard *et al.* (1984), the prolific release of DMS by this alga, which has attracted much attention, is nothing more to the alga itself than a by-product of its protective mechanism.

Antibiosis has been reported from field observations of Antarctic *P. pouchetii* blooms (Burkholder and Sieburth 1961). Bacterial uptake rates increased during the bloom of *P. pouchetii* but peaked as the bloom declined (Laanbroek *et al.* 1985; Lancelot and Billen 1984). This may reflect antibiosis during active growth of *P. pouchetii* but may also be a temperature mediated lag between production and utilization. Billen and Fontigny (1987) claim close linkage between the bacterial biomass and substrate availability from a *P. pouchetii* bloom. Thus, published opinions concerning the effectiveness of the antibiosis vary. However, the bacterial defence mechanism proposed by Davidson and Marchant (1987) may reconcile these opposing views as the bacterial biomass and incorporation rates are dependent on the equilibrium between actively growing and senescent colonies at any stage in the population dynamics. Thus, bacterial numbers and incorporation rates may still be relatively high (Billen and Fontigny 1987) while allowing antibiosis of actively growing colonies.

6.10 *Sea foam*

A few days after the peak of *P. pouchetii* bloom the colonies begin to break up. At this time characteristic development of sea foam is observed, forming long streaks on the open ocean (Eberlein *et al.* 1985; Rogers and Lockwood 1990). The foam results from dissolved organic carbon, composed of proteins and polymeric carbohydrates, released into the water column from decomposition of the massive POC generated during a bloom of *P. pouchetii* (Guillard and Hellebust 1971; Lancelot 1983; Veldhuis *et al.* 1986a). In contrast Eberlein *et al.* (1985) observed close correlation between *P. pouchetii* numbers and the concentration of DOM and concluded that the latter must result from direct exudation. The time of appearance of the sea foam would, however, indicate that the necessary concentrations of dissolved organics are reached only following collapse of the bloom. Blooms of *P. pouchetii* have been observed in the North Sea for a long time but the appearance of foam is a recent phenomenon, having first occurred in 1978 (Bätje and Michaelis 1986). This has been ascribed to the

anthropogenic enrichment of northern European coastal waters (e.g. Bennekom *et al.* 1975) which in turn increases the frequency, abundance and duration of its blooms suggesting that strains of this alga are physiologically adapted to proliferation in nutrient enriched environments (Lancelot *et al.* 1987). Foam deposits reach depths of up to two metres on beaches in the area (Eberlein *et al.* 1985; Bätje and Michaelis 1986; Lancelot *et al.* 1987) being of substantial nuisance value (Lancelot *et al.* 1987). Harpacticoids either passively or actively occupy the foam while appendicularians and nematodes are smothered by it (Armonies 1989).

7. CONCLUSIONS

Phaeocystis pouchetii has become a nuisance alga in the North Sea (Lancelot *et al.* 1987) where the occurrence, abundance and duration of its blooms have increased (e.g. Cadée and Hegeman 1986; Lancelot *et al.* 1987) in response to the anthropogenic enrichment of these coastal waters (e.g. Bennekom *et al.* 1975). Hitherto, few occurrences of *P. pouchetii* have been reported from tropical waters; however, the appearance of blooms of this alga in the Arabian Gulf coincide with nutrient enrichment of these waters by sewage and industries (Al-Hassan *et al.* 1990). Based on its possession of UV-B absorbing compounds, Marchant and Davidson (1991) predict that this species could increase in dominance in Antarctic waters. Thus, it would appear that anthropogenic alteration of the environment enhances the abundance, dominance and distribution of *P. pouchetii* allowing it to display its prominence by forming near monospecific blooms. It remains to be seen whether future human alteration of the marine environment causes *P. pouchetii* increasingly to prosper at the expense of other phytoplankton. If this does occur, ecosystem structure and function in the areas where the species abounds will be strongly influenced by its unique physiology (Lancelot *et al.* 1987; Davidson and Marchant 1992), potentially causing reduced secondary production (e.g. Claustre *et al.* 1990), reduced vertical carbon flux (Marchant and Davidson 1991) and causing changes in the composition of food webs (Lancelot *et al.* 1987; Davidson and Marchant 1992) (Fig. 7).

ACKNOWLEDGEMENTS

We wish to thank Dr Patrick Quilty, Dr Gustaaf Hallegraeff and Deborah Bramich for their critical appraisal of the manuscript and Warren Nicholas for assistance with translating manuscripts.

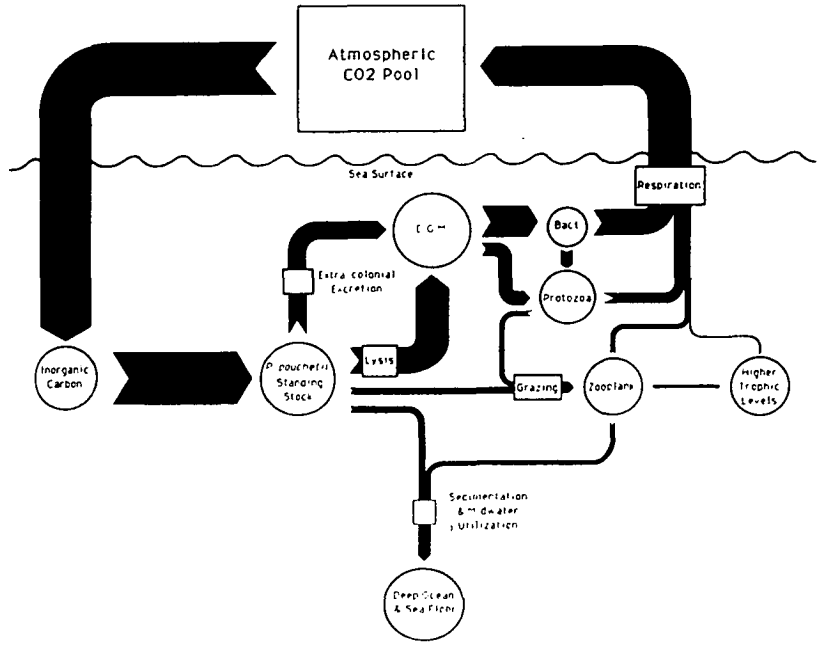


Fig. 7. Conceptual diagram of the contribution made by *P. pouchetii* to the trophic system and carbon flux (after Lancelot *et al.* 1987, Wassmann *et al.* 1990 and Marchant and Davidson 1991).

REFERENCES

Admiraal, W. and Veldhuis, M. J. W. (1987). Determination of nucleosides and nucleotides in seawater by HPLC; application to the phosphatase activity in cultures of the alga *Phaeocystis pouchetii*. *Mar. Ecol. Prog. Ser.* **36**, 277-285.

Admiraal, W. and Venekamp, L. A. H. (1986). Significance of tintinnid grazing during blooms of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. *Neth. J. Sea Res.*, **20**, 61-66.

Ainley, D. G., Fraser, W. R., Sullivan, C. W., Torres, J. J., Hopkins, T. L. and Smith, W. O. (1986). Antarctic mesopelagic micronekton: evidence from seabirds that pack ice affects community structure. *Science*, **232**, 847-849.

Al-Hasan, R. H., Ali, A. M. and Radwan, S. S. (1990). Lipids, and their constituent fatty acids, of *Phaeocystis* sp. from the Arabian Gulf. *Mar. Biol.*, **105**, 9-14.

Andreae, M. O. and Raemdonck, H. (1983). Dimethyl sulfide in the surface ocean and the marine atmosphere: a global view. *Science*, **221**, 744-747.

Armonies, W. (1989). Occurrence of meiofauna in *Phaeocystis* seafoam. *Mar. Ecol. Prog. Ser.*, **53**, 305-309.

Ashworth, T. K., Prasad, A. K. S. K. and Fryxell, G. A. (1990). AMERIEZ 88: Phytoplankton distribution across the Weddell Sea ice edge during austral winter. *Eos*, **71**, 105.

Atkinson, L. P., Paffenhöfer, G.-A. and Dunstan, W. M. (1978). The chemical and biological effect of a Gulf Stream intrusion off St. Augustine, Florida. *Bull. Mar. Sci.*, **28**, 667-679.

Barnard, W. R., Andreae, M. O. and Iverson, R. L. (1984). Dimethylsulfide and *Phaeocystis pouchetii* in the southeastern Bering Sea. *Cont. Shelf. Res.*, **3**, 103-113.

Bates, T. S., Charlson, R. J. and Gammon, R. H. (1987a). Evidence for the climatic role of marine biogenic sulphur. *Nature*, **329**, 319-321.

Bates, T. S., Cline, J. D., Gammon, R. H. and Kelly-Hansen, S R. (1987b). Regional and seasonal variation in the flux of oceanic dimethylsulfide to the atmosphere. *J. Geophys. Res.*, **93**, 2930-2938.

Bätje, M. and Michaelis, H,. (1986). *Phaeocystis pouchetii* blooms in the East Frisian coastal waters (German Bight, North Sea). *Mar. Biol.*, **93**, 21-27.

Baumann, M. E. M. and Jahnke, J. (1986). Marine planktonalgen der Arktis; Die Haptophycee *Phaeocystis pouchetii*. *Mikrokosmos*, **75**, 262-265.

Bennekom, A. J. van, Gieskes, W. W. C. and Tijssen, S. B. (1975). Eutrophication of Dutch coastal waters. *Proc. R. Soc. Lond. (B)*, **189**, 359-374.

Billen, G. and Fontigny, A. (1987). Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. II. Bacterioplankton dynamics. *Mar. Ecol. Prog. Ser.*, **37**, 249-257.

Bjørnland, T., Guillard, R. R. L. and Liaen-Jensen, S. (1988). *Phaeocystis* sp. clone 677-3 - a tropical marine planktonic prymnesiophyte with fucoxanthin and 19'-acyloxyfucoxanthins as chemosystematic carotenoid markers. *Biochem. Syst. Ecol.*, **16**, 445-452.

Boalch, G. T. (1987). Recent blooms in the western English Channel. *Rapp. P. -v. Réun. Cons. Perm. Int. Explor. Mer.* **187**, 94-97.

Boalch, G. T. and Harbour, D. S. (1977). Unusual diatom off the coast of south-west England and its effect on fishing. *Nature*, **269**, 687-688.

Bodungen, B.v., Smetacek, V. S., Tilzer, M. M. and Zeitzschel, B. (1986). Primary production and sedimentation during spring in the Antarctic Peninsula region. *Deep-Sea Res.*, 33, 177-194.

Bölter, M. and Dawson, R. (1982). Heterotrophic utilisation of biocyhemical compounds in antarctic waters. *Neth. J. Sea Res.*, **16**, 315-332.

Booth, B. C., Lewin, J. and Norris, R. E. (1982). Nanoplankton species predominant in the subarctic Pacific in May and June 1978. *Deep-Sea Res.*, **29**, 185-200.

Bougard, M. (1979). Étude bibliographique sur le phytoflagellate *Phaeocystis*. Institut de Biologie Maritime et Regionale de Wimereau. Université des Sciences et Technique de Lille, pp. 1-30.

Bourrelly, P. (1957). Researches sur les Chrysophycees: morphologie, phylogenie, systematique. *Rev. Algol., Mém. H.-Sér*, **1**, 1-42.

Bradstock, M. and Mackenzie, L. (1981). The Tasman Bay slime story. Catch '81 December, 29-30.

Buck, K. R. and Garrison, D. L. (1983). Protists from the ice-edge region of the Weddell Sea. *Deep-Sea Res.*, **30**, 1261-1277.

Bunt, J. S. and Wood, E. J. F. (1986). Microalgae in Antarctic sea ice. *Nature*, **199**, 1254-1255.

Burkholder, P. R. and Sieburth, J. McN. (1961). Phytoplankton and chlorophyll in the Gerlache and Bransfield Straits of Antarctica. *Limnol. Oceanogr.*, **6**, 45-52.

Cadée, G. C. (1982). Tidal and seasonal variation in particulate and dissolved organic carbon in the western Dutch Wadden Sa and Marsdiep tidal inlet. *Neth. J. Sea Res.*, **15**, 228-249.

Cadée, G. C. (1986). Increased phytoplankton primary production in the Marsdiep area (western Dutch Wadden Sea). *Neth. J. Sea Res.*, **20**, 285-290.

Cadée, G. C. (1991). Long-term changes in phytoplankton in marine coastal waters. *J. Phycol.*, **27**, 12.

Cadée, G. C. and Hegeman, J. (1974). Primary production of phytoplankton in the Dutch Wadden Sea. *Neth. J. Sea Res.*, **8**, 240-259.

Cadée, G. C. and Hegeman, J. (1979). Phytoplankton primary production, chlorophyll and composition in an inlet of the western Wadden Sea (Marsdiep). *Neth. J. Sea Res.*, **13**, 224-241.

Cadée, G. C. and Hegeman, J. (1986). Seasonal and annual variation in *Phaeocystis pouchetii* (Haptophyceae) in the westernmost inlet of the Wadden Sea during the 1973 to 1985 period. *Neth. J. Sea Res.*, **20**, 29-36.

Chang, F. H. (1983). The mucilage-producing *Phaeocystis pouchetii* (Prymnesiophyceae), cultured from the 1981 "Tasman Bay slime". *N.Z. J. Mar. Freshwat. Res.*, **17**, 165-168.

Chang, F. H. (1984). The ultrastructure of *Phaeocystis pouchetii* (Haptophyceae) vegetative colonies with special reference to the production of new mucilagenous envelope. *N.Z. J. Mar. Freshwat. Res.*, **18**, 303-308.

Charlson, R. J., Lovelock, J. E., Andreae, M. O. and Warren, S. G. (1987). Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature*, **326**, 655-661.

Chu, S. P. (1946). The utilization of organic phosphorus by phytoplankton. *J. Mar. Biol. Ass. U.K.*, **26**, 285-295

Claustre, H., Poulet, S. A., Williams, R., Marty, J.-C., Coombs, S., Ben Mlih, F., Hapette, A. M. and Martin-Jezequel, V. (1990). A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea. *J. Mar. Biol. Ass. U.K.*, **70**, 197-207.

Codispoti, L. A., Friederich, G. E., Whaling, P. and Friebertshauser, M. E. (1990). Some implications of the nutrient observations made during the 1989 CEAREX experiment. *Eos*, **71**, 79.

Colijn, F. (1983). Primary production in the Ems-Dollard Estuary. (Ph. D. thesis. University of Groningen, pp. 123.

Colijn, F., Villerius, L., Radmaker, M., Hammer, K. D. and Eberlein, K. (1990). Changes in spatial distribution of primary production, photosynthetic pigments and phytoplankton species composition during two surveys in the German Bight. *Neth. J. Sea Res.*, **25**, 155-164.

Dagg, M. J., Vidal, J., Whitledge, T. E., Iverson, R. L. and Goering, J. J. (1982). The feeding, respiration and excretion of zooplankton in the Bering Sea during a spring bloom. *Deep-Sea Res.*, **29**, 45-63.

Daro, M. H. (1985). Field study of selectivity, efficiency and daily variation in the feeding of the marine copepod *Temora longicornis*, in the Southern Bight of the North Sea. *Bull. Mar. Sci.*, **37**, 764.

Davidson, A. T. (1985). Aspects of the biology of *Phaeocystis pouchetii* (Prymnesiophyceae). (Hons. Thesis). University of Tasmania, pp. 1-231.

Davidson, A. T. and Marchant, H. J. (1987). Binding of manganese by the mucilage of Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release. *Mar. Biol.*, **95**, 481-487.

Davidson, A. T. and Marchant, H. J. (1992). Protist abundance and carbon concentration during a *Phaeocystis*-dominated bloom at an Antarctic coastal site. *Polar Biol.*, (in press)

Deprez, P. P., Franzmann, P. D. and Burton, H. R. (1986). Determination of reduced sulfur gases in Antarctic lakes and seawater by gas chromatography after solid absorbant preconcentration. *J. Chromat.*, **362**, 9-21.

Eberlein, K., Leal, M. T., Hammer, K. D. and Hickel, W. (1985). Dissolved organic substances during a *Phaeocystis pouchetii* bloom in the German Bight (North Sea). *Mar. Biol.*, **89**, 311-316.

Eilertsen, H. C. (1989). *Phaeocystis pouchetii* (Hariot) Lagerheim, a key species in Arctic marine ecosystems: Life history and physiology. *Rapp. P.-v. Réun. Cons. Perm. Int. Explor. Mer.*, **188**, 131.

Eilertsen, H. C. and Taasen, J. P. (1984). Investigations on the plankton community of Balsfjorden, Northern Norway: The phytoplankton 1976-1978. Environmental factors, dynamics of growth, and primary production. *Sarsia*, **69**, 1-15.

Eilertsen, H. C., Schei, B. and Taasen, J. P. (1981). Investigations on the plankton community of Balsfjorden, northern Norway: The phytoplankton 1976-1978. Abundance, species composition, and succession. *Sarsia*, **66**, 129-141.

Eilertsen, H. C., Taasen J. P. and Weslawski, J. M. (1989). Phytoplankton studies in the fjords of West Spitzbergen: physical environment and production in spring and summer. *J. Plankt. Res.*, **11**, 1245-1260.

El-Sayed, S. Z., Biggs, D. C. and Holm-Hansen, O. (1983). Phytoplankton standing crop, primary productivity, and near surface nitrogenous nutrient fields in the Ross Sea, Antarctica. *Deep-Sea Res.*, **30**, 871-886.

Estep, K. W. and MacIntyre, F. (1989). Taxonomy, life cycle, distribution and dasmotrophy of *Chrysochromulina*: a theory accounting for scales, haptonema, muciferous bodies and toxicity. *Mar. Ecol. Prog. Ser.*, **57**, 11-21.

Estep, K. W., Davis, P. G., Hargraves, P. E. and Sieburth, J. McN. (1984). Chloroplast containing microflagellates in natural populations of North Atlantic nanoplankton, their identification and distribution; including a description of five new species of *Chrysochromulina* (Prymnesiophyceae). *Protistologica*, **20**, 613-634.

Estep, K. W., Nejstgaard, J. C., Skjoldal, H. R. and Rey, F. (1990). Predation of copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. *Mar. Ecol. Prog. Ser.*, **67**, 235-249.

Foster, P., Voltolina, D., Spencer, C. P., Miller, I. and Beardall, J. (1983). A seasonal study of the distribution of surface state variables in Liverpool Bay. V. Summer. *J. Exp. Mar. Biol. Ecol.*, **73**, 151-165.

Fretter, V. and Montgomery, M. C. (1968). The treatment of food by prosobranch veligers. *J. Mar. Biol. Ass. U.K.*, **48**, 499-520.

Fryxell, G. A. (1989). Marine phytoplankton at the Weddell Sea ice edge: seasonal changes at the specific level. *Polar Biol.*, **10**, 1-18.

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

Fryxell, G. A., Theriot, E. C. and Buck, K. R. (1984). Phytoplankton, ice algae, and choanoflagellates from AMERIEZ, the southern Atlantic and Indian Oceans. *Ant. J. U.S.*, **19**, 1017-109.

Gabbot, P. A. and Walker, A. J. M. (1971). Changes in the condition index and biochemical content of adult oyster (*Ostrea edulis* L.) mantained under hatchery conditions. *J. Cons. Perm. Int. Explor. Mer.*, **34**, 99-106.

Garrison, D. L. and Buck, K. R. (1989). The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsula regions. *Polar Biol.*, **10**, 211-219.

Garrison, D. L., Buck, K. R. and Fryxell, G. A. (1987). Algal assemblages in Antarctic pack ice and the ice-edge plankton. *J. Phycol.*, **23**, 564-572.

Gibson, J. A. E., Garrick, R. C., Burton, H. R. and McTaggart, A. R. (1990). Dimethylsulfide and the alga *Phaeocystis pouchetii* in antarctic coasal waters. *Mar. Biol.*, **104**, 339-346.

Gieskes, W. W. C. and Bennekom, A. J. v. (1973). Unreliability of the ^{14}C method for estimating primary production in Dutch coastal waters. *Limnol. Oceanogr.*, **18**, 494-495.

Gieskes, W. W. C. and Kraay, G. W. (1975). The phytoplankton spring bloom in Dutch coastal waters of the North Sea. *Neth. J. Sea. Res.*, **9**, 166-196.

Gieskes, W. W. C. and Kraay, G. W. (1977). Continuous plankton records: changes in the plankton of the North Sea and its eutrophic Southern Bight from 1948 to 1975. *Neth. J. Sea Res.*, **11**, 334-364.

Gieskes, W. W. C. and Kraay, G. W. (1986). Analysis of phytoplankton pigments by HPLC before, during and after mass occurrence of the flagellate *Corymbellus aureus* during the spring bloom in the open northern North Sea in 1983. *Mar. Biol.*, **92**, 45-52.

Gran, H. H. (1902). Das plankton des Norwegischen Nordmeers von biolischen Gesichtspunkten behandelt. *Rep. Norweg. Fish. Mar. Inv.*, **2**, 1-222.

Gran, H. H. (1929). Investigation of the production of phytoplankton outside the Romsdalsfjord 1926-1927. *Rapp. P.-v. Réun. Cons. Perm. Int. Explor. Mer.*, **56**, 1-112.

Gran, H. H. (1930). The spring growth of the phytoplankton at Møre in 1928-29 and at Lofoten in 1929 in relation to its limiting factors. *Det Norske-Vidensk. Akad. Oslo, I. Mat. Naturv. Kl*, **5**, 1-77.

Green, J. C. (1976). *Corymbellus aureus* gen. et sp. nov., a new colonial member of the Haptophyceae. *J. Mar. Biol. Ass. U.K.*, **56**, 31-38.

Green, J. C. (1962). Biosynthesis of Dimethyl-β-propiothetin. *J. Biol. Chem.*, **237**, 2251-2254.

Grimm, N. and Weisse, T. (1985). Die temperaturabhängigkeit des wachstums von *Phaeocystis pouchetii* (Haptophyceae) in batchkulturen. *Helgoländer Wiss. Meeresunters.*, **39**, 201-211.

Grontved, J. (1960). Planktological contribution IV. Taxonomic and productional investigatiions in shallow coastal waters. *Meddr. Kommn. Danm. Fisk. -og Havunder.*, **3**, 1-17.

Guillard, R. R. L. and Hellebust, J. A. (1971). Growth and the production of extracellular substances by two strains of *Phaeocystis pouchetii*. *J. Phycol.*, **7**, 330-338.

Gunkel, W. (1982). Mikrobiologisch-chemische untersuchungen an meeresschaum. Jahresber. 1981. *Biol. Anst. Helgoländ*, pp. 49-50.

Haekel, E. (1890). Plankton-stidien. Vergleichende Untersuchungen über die Bedeutung und Zusammensetzung der pelagischen fauna and flora. *Jena Ztschr. Naturwiss.*, **25**, 232-236.

Hallegraeff, G. M. (1983). Scale-bearing and loricate nanoplankton from the East Australian current. *Bot. Mar.*, **26**, 493-515.

Hansen, B., Tande, K. S. and Berggreen, U. C. (1990). On the trophic fate of *Phaeocystis pouchetii* (Hariot). III. Functional responses in grazing demonstrated on juvenile stages of *Calanus finmarchicus* (Copepoda) fed diatoms and *Phaeocystis*. *J. Plankton Res.*, **12**, 1173-1187.

Hart, T. J. (1942). Phytoplankton periodicity in Antarctic surface waters. *Disc. Rep.*, **21**, 261-356.

Hatekeyama, S., Izumi, K. and Akimoto, H. (1985). Yield of SO_2 and formation of aerosol in the photo-oxudation of DMS under atmospheric conditions. *Atmosph. Environ.*, **19**, 135-141.

Hickel, W. (1984). Seston in the Wadden Sea of Sylt (German Bight, North Sea). *Neth. Inst. Sea Res. Publ. Ser.*, **10**, 113-131

Holligan, P. M. (1987). The physical environment of exceptional phytoplankton blooms in the northeast Atlantic. *Rapp. P.-v. Réun. Cons. Perm. Int. Explor. Mer.*, **187**, 9-18.

Hollowday, E. D. (1949). A preliminary report on the Plymouth marine and brackish-water rotifer. *J. Mar. Biol. Ass. U.K.*, **28**, 239-254.

Holm-Hansen, O., Mitchell, B. G., Hewes, C. D. and Karl, D. M. (1989). Phytoplankton blooms in the vicinity of Palmer Station, Antarctica. *Polar. Biol.*, **10**, 49-57.

Huntley, M., Tande, K. and Eilertsen, H. C. (1987). On the trophic fate of *Phaeocystis pouchetii* (Hariot). II. Grazing rates of *Calanus hyperboreus* feeding on diatoms and different size categories of *P. pouchetii*. *J. Exp. Mar. Biol. Ecol.*, **110**, 197-212.

Iverson, R. L., Whitledge, T. E. and Goering, J. J. (1979). Chlorophyll and nitrate fine structure in the southeastern Bering Sea shelf break front. *Nature*, **281**, 664-666.

Jahnke, J. (1989). The light and temperature dependence of growth rate and elemental composition of *Phaeocystis globosa* Scherffel and *P. pouchetii* (Har.) Lagerh. in batch cultures. *Neth. J. Sea Res.*, **23**, 15-21.

Jahnke, J. and Baumann, M. E. M. (1986). Die marine planktonalge *Phaeocystis globosa*; Eine massenform unserer küstengewässer. *Mikrokosmos*, **75**, 357-359.

Jahnke, J. and Baumann, M. E. M. (1987). Differenciation between *Phaeocystis pouchetii* (Har.) Lagerheim and *Phaeocystis globosa* Scherffel. I. Colony shapes and temperature tolerances. *Hydrobiol. Bull.*, **21**, 141-147.

Jeffrey, S. W. and Hallegraeff, G. M. (1987). Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. *Mar. Ecol. Prog. Ser.*, **35**, 293-304.

Joint, I. R. and Pomroy, A. J. (1981). Primary production in a turbid estuary. *Estuar. Cstl. Shelf Sci.*, **13**, 303-316.

Joiris, C., Billen, G., Lancelot, C., Daro, M. H., Mommaerts, J. P., Bertels, A., Bossicart, M., Nijs, J. and Hecq, J. H. (1982). A budget of carbon cycling in the Belgian coastal zone: relative role of zooplankton, bacterioplankton and benthos in the utilisation of primary production. *Neth. J. Sea Res.*, **16**, 260-275.

Jones, P. G. W. and Haq, S. M. (1963). The distribution of *Phaeocystis* in the eastern Irish Sea. *J. Cons. Perm. Int. Explor. Mer.*, **28**, 8-20.

Jones, M. and Spencer, P. C. (1970). The phytoplankton of the Menai Straits. *J. Cons. Perm. Int. Explor. Mer.*, **33**, 169-180.

Kashkin, N. I. (1963). Materials on the ecology of *Phaeocystis pouchetii* (Hariot) Lagerheim, 1893 (Chrysophyceae). II. Habitat and specifications of biogeographical characteristics. *Okeanologiya*, **3**, 697-705.

Kayser, H. (1970). Experimental-ecological investigations on *Phaeocystis pouchetii* (Haptophyceae): cultivation and waste water test. *Helgoländer Wiss. Meeresunters.*, **20**, 195-212.

Keller, M. D., Bellows, W. K. and Guillard, R. R. L. (1989). Dimethyl sulfide production in marine phytoplankton. In: *Biogenic sulfur in the environment* (Saltzmann, E. S. and Cooper, W. J., eds), pp. 1657-182. ACS Symposium Series 393. American Chemical Society, Washington D.C.

Kommann, P. (1955). Beobachtungen an *Phaeocystis*-Kulturen. *Helgoländer Wiss. Meeresunters.*, **5**, 218-233.

Künne, C. (1952). Untersuchungen über das grossplankton in der Deutschen Bucht und im Nordsylter Wattenmeer. *Helgoländer Wiss. Meeresunters.*, **4**, 1-54.

Laanbroek, H. J., Verplanke, J. C., de Visscher, P. R. M. and de Vuyst, R. (1985). Distribution of phyto- and bacterioplankton growth and biomass parameters, dissolved inorganic nutrients and free amino acids during a spring bloom in the Oosterschelde basin, The Netherlands. *Mar. Ecol. Prog. Ser.*, **25**, 1-11.

Lagerheim, G. (1893). *Phaeocystis*, nov. gen., grundadt på *Tetraspora Poucheti* Har. *Bot. Notiser*, **1**, 32-33.

Lagerheim, G. (1896). Ueber *Phaeocystis pouchetii* (Hariot) Lagberheim, eine plankton-flagellate. *Öefvers. Kongl. Svenska Vet. Akad. Forhandl.*, **53**, 277-288.

Lancelot, C. (1983). Factors affecting phytoplankton extracellular release in the Southern Bight of the North Sea. *Mar. Ecol. Prog. Ser.*, **12**, 115-121.

Lancelot, C. (1984a). Extracellular release of small and large molecules by phytoplankton in the southern bight of the North Sea. *Estuar. Cstl. Shelf Sci.*, **18**, 65-77.

Lancelot, C. (1984b). Metabolic changes in *Phaeocystis poucheti* (Hariot) Lagerheim during the spring bloom in Belgian coastal waters. *Estuar. Cstl. Shelf Sci.*, **18**, 593-600.

Lancelot, C. and Billen, G. (1984). Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. *Limnol. Oceanogr.*, **29**, 721-730.

Lancelot, C. and Mathot, S. (1985). Biochemical fractionation of primary production of phytoplankton in Belgian coastal waters during short- and longterm incubation with ^{14}C-bicarbonate. II. *Phaeocystis pouchetii* colonial population. *Mar. Biol.*, **86**, 227-232.

Lancelot, C. and Mathot, S. (1987). Dynamics of a *Phaeocystis*-dominated spring bloom in Belgian coastal waters. I. Phytoplankton activity and related parameters. *Mar. Ecol. Prog. Ser.*, **37**, 239-248.

Lancelot, C., Mathot, S. and Owens, N. J. P. (1986). Modelling protein synthesis, a step to an accurate estimate of net primary production: *Phaeocystis pouchetii* colonies in Belgian coastal waters. *Mar. Ecol. Prog. Ser.*, **32**, 193-202.

Lancelot, C., Billen, G., Sournia, A., Weisse, T., Colijn, F., Veldhuis, M. J. W., Davies, A. and Wassmann, P. (1987). *Phaeocystis* blooms and nutrient enrichment in the continental coastal zones of the North Sea. *Ambio*, **16**, 38-46.

Lebour, M. V. (1922). The food of plankton organisms. *J. Mar. Biol. Ass. U.K.*, **12**, 644-677.

Legendre, L. (1990). The significance of microalgal blooms for fisheries and for the export of particulate organic carbon in oceans. *J. Plankton Res.*, **12**, 681-699.

Lovelock, J. E., Maggs, R. J. and Rasmussen, R. A. (1972). Atmospheric dimethyl sulphide and the natural sulphur cycle. *Nature*, **237**, 418-420.

Lubbers, G. W., Gieskes, W. W. C., del Castilho, P., Salomons, W. and Bril, J. (1990). Manganese accumulation in the high pH microenvironment of *Phaeocystis* sp. (Haptophyceae) colonies from the North Sea. *Mar. Ecol. Prog. Ser.*, **59**, 285-293.

Lucas, C. E. (1940). Ecological investigations with the continuous plankton recorder: the phytoplankton in the southern North Sea, 1932-1937. *Hull Bull. Mar. Ecol.*, **1**, 73-170.

Lutter, S., Taasen, J. P., Hopkins, C. C. E. and Smetacek, V. (1989). Phytoplankton dynamics and sedimentation processes during spring and summer in Balsfjord, Northern Norway. *Polar Biol.*, **10**, 113-124.

Marchant, H. J. and Davidson, A. T. (1991). Possible impacts of ozone depletion on trophic interactions and biogenic vertical carbon flux in the Southern Ocean. In: *Proceedings of the International Conference on the Role of Polar Regions in Global Change* (Weller, G., ed.). Fairbanks, in press.

Marchant, H. J., Davidson, A. T. and Kelly, G. (1991). UV-B protecting pigments in the alga *Phaeocystis pouchetii* from Antarctica. *Mar. Biol.*, **109**, 391-395.

Marchant, H. J. and Nash, G. V. (1986). Electron microscopy of gut contents and faeces of *Euphausia superba* Dana. *Mem. Natl. Inst. Polar Res., Spec. Issue*, **40**, 167-177.

Margalef, R. (1978). The phytoplankton communities in upwelling areas. The example of NW Africa. *Oecologia Aquatica*, **3**, 97-132.

Marr, J. (1962). The natural history and geography of Antarctic krill (*Euphausia superba* Dana). *Disc. Rep.*, **32**, 33-464.

Martens, P. (1980). Beitrage zum mesozooplankton des Nordsylter Wattenmeeres. *Helgoländer Wiss. Meeresunters.*, **34**, 41-53.

Martens, P. (1981). On the *Acartia* species in the northern Wadden Sea of Sylt. *Kieler Meeresforsch. Sonderh.*, **5**, 153-163.

Meyer, M. A. and El-Sayed, S. Z. (1983). Grazing of *Euphausia superba* Dana on natural phytoplankton populations. *Polar Biol.*, **1**, 193-197.

Miller, D. G. M. and Hampton, I. (1989). Biology and ecology of Antarctic krill (*Euphausia superba* Dana). *Biomass Scientific Series*, **9**, 1-166.

Moestrup, Ø. (1979). Identification by electron microscopy of marine nanoplankton from New Zealand, including the description of four new species. *N.Z. J. Bot.*, **17**, 61-95.

Mommaerts, J. P. (1973). The relative importance of nanoplankton in the North Sea primary production. *Br. phycol. J.*, **8**, 13-20.

Morel, F. M. M., Reuter, J. G., Anderson, D. M. and Guillard, R. R. L. (1979). Aquil: a chemical defined phytoplankton culture medium for trace metal studies. *J. Phycol.*, **15**, 135-141.

Morris, A. W. (1971). Trace metal variations in sea water of Menai Straits caused by a bloom of *Phaeocystis*. *Nature*, **233**, 427-428.

Morris, I., Glover, H. E. and Yentsch, C. S. (1974). Products of photosynthesis by marine phytoplankton: the effects of environmental factors on the relative rates of protein synthesis. *Mar. Biol.*, **27**, 1-9.

Nichols, P. D., Skerratt, J. H., Davidson, A., Burton, H. and McMeekin, T. A. (1991). The lipid composition of cultured *Phaeocystis pouchetii*: Signatures for food-web, biogeochemical and environmental studies in Antarctica and the Southern Ocean. *Phytochemistry*, **30**, 3209-3214.

Nicholls, A. G. (1935). The larval stages of *Longipedia coronata* Claus, *L. scotti* G. O. Sars, and *L. minor* T. and A. Scott, with a description of the male *L. scotti*. *J. Mar. Biol. Ass. U.K.*, **20**, 29-35.

Nøst-Hegseth, E. (1982). Chemical and species composition of the phytoplankton during the first spring bloom in Trondheimsfjorden, 1975. *Sarsia*, **67**, 131-141.

O'Kelly, J. C. (1974). Inorganic nutrients. In: *Algal Physiology and Biochemistry* (Stewart, W. D. P., ed.), 610-635. Blackwell Scientific Publications, Oxford.

Orton, J. H. (1923). The so-called "baccy-juice" in the waters of the Thames oyster-beds. *Nature*, **111**, 773.

Owens, N. J. P., Cook, D., Colebrook, M., Hunt, H. and Reid, P,. C. (1989). Long term trends in the occurrence of *Phaeocystis* sp. in the north-east Atlantic. *J. Mar. Biol. Ass. U.K.*, **69**, 813-821.

Paerl, H. W. (1988). Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. *Limnol. Oceanogr.*, **33**, 823-847.

Pain, S. (1989). Pollutants collect on continental side of North Sea, study finds. *New Scientist*, **11**, 24.

Palmisano, A. C. and Sullivan, C. W. (1985). Pathways of photosynthetic carbon assimilation in sea-ice microalgae from McMurdo Sound, Antarctica. *Limnol. Oceanogr.*, **30**, 674-678.

Palmisano, A. C., SooHoo, J. B., SooHoo, S. L., Kottmeier, S. T., Craft, L. L. and Sullivan, C. W. (1986). Photoadaptation in *Phaeocystis pouchetii* advected beneath annual sea ice in McMurdo Sound, Antarctica. *J. Plankton Res.*, **8**, 891-906.

Parke, M. and Dixon, P. S. (1968). Check-list of British marine algae - 2nd revision. *J. Mar. Biol. Ass. U.K.*, **48**, 783-832.

Parke, M., Green, J. C. and Manton, I. (1971). Observations on the fine structure of the zoids of the genus *Phaeocystis* (Haptophyceae). *J. Mar. Biol. Ass. U.K.*, **51**, 927-941.

Pearce, F. (1988). Plankton shares the blame for sulphur pollution. *New Scientist*, **117**, 25.

Perrin, R. A., Lu, P. and Marchant, H. J. (1987). Seasonal variation in marine phytoplankton and ice algae at a shallow Antarctic coastal site. *Hydrobiologia*, **146**, 33-46.

Pienaar, R. N. and Cooper, G. A. (1991). Ultrastructure of the motile disc-bearing phase of *Phaeocystis*. *J. Phycol.*, **27**, 59.

Pieters, H., Kluytmans, J. H., Zandee, D. I. and Cadée, G. C. (1980). Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. *Neth. J. Sea Res.*, **14**, 349-361.

Pouchet, M. G. (1892). Sur une algue pélagique nouvelle. *Compte Rendus Hebdomadaires Seances et Memoires Societe de Biol.*, **44**, 34-36.

Priscu, J. C., Priscu, L. R., Palmisano, A. C. and Sullivan, C. W. (1990). Estimation of neutral lipid levels in Antarctic sea ice microalgae by nile red fluorescence. *Antarctic Science*, **2**, 149-155.

Reid, P. C. (1975). Large scale changes in North Sea phytoplankton. *Nature*, **257**, 217-219.

Reynolds, C. S., Thompson, J. M., Ferguson, A. J. D. and Wiseman, S. W. (1982). Loss processes in the population dynamics of phytoplankton maintained in closed systems. *J. Plankton Res.*, **4**, 561-600.

Richardson, M. G. and Whitaker, T. M. (1979). An antarctic fast-ice food chain: Observations on the interaction of the amphipod *Pontogeneia antarctica* Chevreux with ice-associated micro-algae. *Br. Antarc. Surv. Bull.*, **47**, 107-115.

Rick, H.-J. and Aletsee, L. (1989). The distribution of the haptophytes *Phaeocystis pouchetii* (Hariot) Lagerheim and *Phaeocystis globosa* Scherffel in the North Sea during May, June 1986 and February, March 1987. *Meeresforsch.*, **32**, 169-176.

Riegman, R., Colijn, F., Malschaert, J. F. P., Kloosterhuis, H. T. and Cadée, G. C. (1990). Assessment of growth rate limiting nutrients in the North Sea by the use of nutrient-uptake kinetics. *Neth. J. Sea Res.*, **26**, 52-60.

Rijswijk, P.v., Bakker, C. and Vink, M. (1989). Daily fecundity of *Temora longicornis* (Copepoda Calanoida) in the Oosterschelde estuary (SW Netherlands). *Neth. J. Sea Res.*, **23**, 293-303.

Rogers, S. I. and Lockwood, S. J. (1990). Observations on coastal fish fauna during a spring bloom of *Phaeocystis pouchetii* in the eastern Irish Sea. *J. Mar Biol. Ass. U.K.*, **70**, 249-253.

Rousseau, V., Mathot, S. and Lancelot, C. (1990). Calculating carbon biomass of *Phaeocystis* sp. from microscopic observations. *Mar. Biol.*, **197**, 305-314.

Sargent, J. R. and Falk-Petersen, S. (1989). The lipid biochemistry of *Calanus*. *Hydrobiologia*, **167/168**, 101-114.

Sargent, J. R., Eilertsen, H. C., Falk-Petersen, S. and Taasen, J. P. (1985). Carbon assimilation and lipid production in phytoplankton in the northern Norwegian fjords. *Mar. Biol.*, **85**,109-116.

Savage, R. E. (1930). The influence of *Phaeocystis* on the migrations of the herring. *Fish. Invest. Ser. 2*, **12**, 1-14.

Savage, R. E. (1932). *Phaeocystis* and herring shoals. *J. Ecol.*, **20**, 326-340.

Scherffel, A. (1899). *Phaeocystis globosa* n. sp. (Vorläufige Mitteilung). *Ber. dt. Bot. Ges.*, **17**, 317-318.

Scherffel, A. (1900). *Phaeocystis globosa* nov. spec. nebst einegen betrachtungen über die phylogenie niederer, insbesonderer brauner organismen. *Wiss. Meeresunters. NF Abt. Helgoländ*, **4**, 1-28.

Schnack, S. B. (1983). On the feeding of copepods on *Thalassiosira partheneia* from the northwest African upwelling area. *Mar. Ecol. Prog. Ser.*, **11**, 49-53.

Sieburth, J. McN. (1960). Acrylic acid, an "antibiotic" principal in *Phaeocystis* blooms in Antarctic waters. *Science*, **132**, 676-677.

Sieburth, J. McN. (1961). Antibiotic properties of acrylic acid. A factor in gastrointestinal antibiosis of polar marine animals. *J. Bacteriol.*, **82**, 72-79.

Sieburth, J. McN. (1964). Antibacterial substances produced by marine algae. In: *Developments in Industrial Microbiology*, pp. 124-134. Soc. Industr. Microbiol., Washington, D.C.

Sieburth, J. McN. (1979). *Sea Microbes*. 491pp. Oxford University Press, London.

Smayda, T. J. (1973). The growth of *Skeletonema costatum* during a winter-spring bloom in Narragansett Bay, R. I. *Norw. J. Bot.*, **20**, 219-247.

Smayda, T. J. (1980). Phytoplankton species succession. In: *The Physiological Ecology of Phytoplankton* (Morris, I. O., ed.), 493-570. Blackwell Scientific Publications, London

Smith, W. O., Jr. and Nelson, D. M. (1986). Importance of ice edge phytoplankton blooms in the Southern Ocean. *BioScience*, **36**, 251-257.

SooHoo, J. B., Palmisano, A. C., Kottmeier, S. T., Lizotte, M. P., SooHoo, S. L. and Sullivan, C. W. (1987). Spectral light absorbance and quantum yield of photosynthesis in sea ice microalgae and a bloom of *Phaeocystis pouchetii* from McMurdo Sound, Antarctica. *Mar. Ecol. Prog. Ser.*, **39**, 175-189.

Sournia, A. (1988). *Phaeocystis* (Prymnesiophyceae): How many species? *Nova Hedwigia*, **47**, 211-217.

Stefánsson, U. and Ólafsson, J. (1990). Anomalous silicate-nitrate relationships associated with *Phaeocystis pouchetii* blooms. *Eos*, **71**, 66.

Stolarski, R. S., Krueger, A. J., Schoeberl, M. R., McPeters, R. D., Newman, P. A. and Alpert, J. C. (1986). Nimbus 7 satellite measurements of the springtime Antarctic ozone decrease. *Nature*, **322**, 808-811.

Tande, K. S. and Båmstedt, U. (1987). On the trophic fate of *Phaeocystis pouchetii*. I. Copepod feeding rates on solitary cells and colonies of *P. pouchetii*. *Sarsia*, **72**, 313-320.

Vairavamurthy, A., Andreae, M. O. and Iverson, R. L. (1985). Biosynthesis of dimethylsulfide and dimethylpropiothetin by *Hymenomonas carterae* in relation to the sulfur source. *Limnol. Oceanogr.*, **303**, 59-70.

Veldhuis, M. J. W. (1987). The eco-physiology of the colonial alga *Phaeocystis pouchetii*. Rijksuniv. Groningen Doct.

Veldhuis, M. J. W. and Admiraal, W. (1985). Transfer of photosynthetic products in gelatinous colonies of *Phaeocystis pouchetii* (Haptophyceae) and its effect on the measurement of excretion rate. *Mar. Ecol. Prog. Ser.*, **26**, 301-304.

Veldhuis, M. J. W. and Admiraal, W. (1987). Influence of phosphate depletion on the growth and colony formation of *Phaeocystis pouchetii*. *Mar. Biol.*, **95**, 47-54.

Veldhuis, M. J. W., Admiraal, W. and Colijn, F. (1986a). Chemical and physiological changes of the phytoplankton during the spring bloom, dominated by *Phaeocystis pouchetii* (Haptophyceae): Observation in Dutch coastal waters of the North Sea. *Neth. J. Sea Res.*, **20**, 49-60.

Veldhuis, M. J. W., Colijn, F. and Admiraal, W. (1991). Phosphate utilization in *Phaeocystis pouchetii* (Haptophyceae). *Mar. Ecol.*, **12**, 53-62.

Veldhuis, M. J. W., Colijn, F. and Venekamp, L. A. H. (1986b). The spring bloom of *Phaeocystis pouchetii* (Haptophyceae) in Dutch coastal waters. *Neth. J. Sea Res.*, **20**, 37-48.

Veldhuis, M. J. W., Venekamp, L. A. H. and Ietswaart, T. (1987). Availability of phosphorus sources for blooms of *Phaeocystis pouchetii* (Haptophyceae) in the North Sea: impact of the River Rhine. *Neth. J. Sea Res.*, **21**, 219-229.

Veldhuis, M. J. W., Colijn, F., Venekamp, L. A. H. and Villerius, L. (1988). Phytoplankton primary production and biomass in the western Wadden Sea (The Netherlands); a comparison with an ecosystem model. *Neth. J. Sea Res.*, **22**, 37-49

Verity, P. G. and Smayda, T. J. (1989). Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): ingestion, egg production and growth of nauplii. *Mar. Biol.*, **100**, 161-171.

Verity, P. G., Villareal, T. A. and Smayda, T. J. (1988a). Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. I. Abundance, biochemical composition, and metabolic rates. *J. Plankton Res.*, **10**, 219-248.

Verity, P. G., Villareal, T. A. and Smayda, T. J. (1988b). Ecological investigations of blooms of colonial *Phaeocystis pouchetii*. II. The role of life-cycle phenomena in bloom termination. *J. Plankton Res.*, **10**, 749-766.

Vesk, M. and Jeffrey, S. W. (1987). Ultrastructure and pigments of two strains of the picoplanktonic alga *Pelagococcus subviridis* (Crysophyceae). *J. Phycol.*, **23**, 322-336.

Walne, P. R. (1970). Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria*, *and Mytlius*. *Fish. Invest.*, London, **26**, 1-62.

Wassmann, P., Vernet, M., Mitchell, B. G. and Rey, F. (1990). Mass sedimentation of *Phaeocystis pouchetii* in the Barents Sea. *Mar. Ecol. Prog. Ser.*, **66**, 183-195.

Weisse, T. (1983). Feeding of calanoid copepods in relation to *Phaeocystis pouchetii* blooms in the German Wadden Sea area off Sylt. *Mar. Biol.*, **74**, 87-94.

Weisse, T. and Scheffel-Möser, U. (1990). Growth and grazing loss rates in single-celled *Phaeocystis* sp. (Prymnesiophyceae). *Mar. Biol.*, **106**, 153-158.

Weisse, T., Grimm, N., Hickel, W. and Martens, P. (1986). Dynamics of *Phaeocystis pouchetii* blooms in the Wadden Sea of Sylt (German Bight, North Sea). *Estuar. Cstl. Shelf Sci.*, **23**, 171-182.

Wright, S. W. and Jeffrey, S. W. (1987). Fucoxanthin pigment markers of marine phytoplankton analysed by HPLC and HPTLC. *Mar. Ecol. Prog. Ser.*, **38**, 259-266.

Wulff, A. (1934). Über hydrographie und oberflächenplankton nebst verbreitung von *Phaeocystis* in der DeutschenBucht im Mai 1933. *Ber. Dt. Wiss. Kommn. Meeresforsch.*, **7**, 343-350.

Yin, F., Grosjean, D. and Seinfeld, J. H. (1986). Analysis of atmospheric photooxidation mechanisms for organic sulfur compounds. *J. Geophys. Res.*, **91**, 14417-14438.

Possible Impacts of Ozone Depletion on Trophic Interactions and Biogenic Vertical Carbon Flux in the Southern Ocean

Harvey J. Marchant and Andrew Davidson
Australian Antarctic Division, Kingston, Tasmania, Australia

ABSTRACT

Among the most productive region of the Southern Ocean is the marginal ice edge zone that trails the retreating ice edge in spring and early summer. The timing of this near-surface phytoplankton bloom coincides with seasonal stratospheric ozone depletion when UV irradiance is reportedly as high as in mid-summer. Recent investigations indicate that antarctic marine phytoplankton are presently UV stressed. The extent to which increasing UV radiation diminishes the ability of phytoplankton to fix CO_2 and/or leads to changes in their species composition is equivocal. The colonial stage in the life cycle of the alga *Phaeocystis pouchetii* is one of the major components of the bloom. We have found that this alga produces extracellular products which are strongly UV-B absorbing. When exposed to increasing levels of UV-B radiation, survival of antarctic colonial *Phaeocystis* was significantly greater than colonies of this species from temperate waters and of the single-celled stage of its life cycle which produces no UV-B-absorbing compounds. *Phaeocystis* is apparently a minor dietary component of antarctic krill, *Euphausia superba*, and its nutritional value to crustacea is reportedly low. Phytoplankton, principally diatoms, together with fecal pellets and molted exoskeletons of grazers contribute most of the particulate carbon flux from the euphotic zone to deep water. If the species composition of antarctic phytoplankton was to shift in favor of *Phaeocystis* at the expense of diatoms, changes to pelagic trophic interactions as well as vertical carbon flux are likely.

INTRODUCTION

Stratospheric ozone over Antarctica and the Southern Ocean is markedly depleted during spring [Stolarski et al., 1986], resulting in UV flux rates similar to mid-summer conditions [Frederick and Snell, 1988]. Solar UV-B radiation penetrates oceanic water to depths that are able to influence the growth of macrophytes and phytoplankton [Jitts et al., 1976; Lorenzen, 1979; Calkins and Thordardottir, 1980; Worrest, 1983; Maske, 1984; Wood, 1987, 1989]. In addition, Trodahl and Buckley [1989] suggest that antarctic sea ice in early spring may be sufficiently transparent to UV for organisms living in and under it to receive levels of radiation high enough to have biological consequences.

The effect of UV-B radiation on antarctic marine phytoplankton is equivocal [Roberts, 1989]. El-Sayed et al. [1990] concluded that antarctic phytoplankton are presently under UV stress and are likely to be seriously affected by any increase in UV-B radiation as would the higher trophic levels of the Southern Ocean food web. In contrast, Holm-Hansen et al. [1989] found that although the rate of photosynthesis by phytoplankton in the top meter of the water column was depressed by about 30%, organisms at depths greater than 20 m were unaffected by *in situ* exposure to UV. They concluded that increased UV irradiation would have little impact on the phytoplankton and higher trophic levels of the Southern Ocean. Species of phytoplankton differ in their ability to survive UV irradiation [Calkins and Thordardottir, 1980], and Karentz [1991] has argued that the most likely effect of elevated UV irradiation on antarctic marine phytoplankton is a shift in the species composition.

The principal primary producers in the Southern Ocean are diatoms. As well as contributing directly to the vertical flux of carbon, they are grazed by crustacea, especially euphausiids. The feces and molted exoskeletons of grazers constitute a major avenue of carbon to deep water [Nicol and Stolp, 1989]. Here we briefly review the spatial and temporal distribution of antarctic marine phytoplankton, especially *Phaeocystis pouchetii*. We discuss our finding of UV-B-absorbing pigments in this alga and the protection that they confer [Marchant et al., 1991] and consider the possible consequences on trophic interactions and biogenic vertical carbon flux of *Phaeocystis* surviving elevated levels of UV exposure.

SPRINGTIME SEA ICE RETREAT AND THE MARGINAL ICE EDGE ZONE

Meltwater released from the retreating sea ice generates a pycnocline at about 20 m depth above which phytoplankton bloom. Data from Jennings et al. [1984] indicate that 25–67% of the nutrient depletion in the Southern Ocean is due to phytoplankton production in the marginal ice edge zone [Smith and Nelson, 1986]. This southward-moving region of high productivity is coupled to higher trophic levels [Ainley et al., 1986], providing much of the carbon required to sustain the large populations of zooplankton, birds and mammals for which the Southern Ocean is noted [Ross and Quetin, 1986; Sakshaug and Skjoldal, 1989].

The most abundant components of the phytoplankton of the marginal ice edge zone are diatoms, principally of the genus *Nitzschia*, and the prymnesiophyte *Phaeocystis pouchetii* [Garrison et al., 1987; Fryxell and Kendrick, 1988; Garrison and Buck, 1989; Davidson and Marchant, 1991]. The massive deposits of diatomaceous ooze in Southern Ocean sediments, the species composition of which is dominated by the taxa found in the ice edge bloom [Truesdale and Kellogg, 1979] are thought to be due to reduced coupling of production and consumption in the marginal ice edge zone. Thus a substantial amount of the biogenic production sinks rapidly from the euphotic zone [Smith and Nelson, 1986] and while some is grazed, sedimentation is apparently the principal fate of much of this ice edge bloom [Smith and Nelson, 1986; Bodungen et al., 1986; Fischer et al., 1988].

THE ROLE OF *PHAEOCYSTIS* IN THE MARGINAL ICE EDGE ZONE

The cosmopolitan alga *Phaeocystis pouchetii* has two principal stages in its life cycle, free-swimming biflagellate unicells and a colonial phase in which cells are embedded in a mucilaginous matrix. Colonial *Phaeocystis* has been reported from the sea ice and the marginal ice edge zone where it is frequently one of the most abundant algae blooming in the top few meters of the water column. *Phaeocystis* apparently plays a pivotal role in the timing of the successional sequence of other autotrophs by mediating the availability of manganese [Davidson and Marchant, 1987; Lubbers et al., 1989]. Also, at least in antarctic waters, this alga provides substrates for heterotrophs by secretion of a large proportion of its photoassimilated carbon as particulate and dissolved organic matter [Davidson and Marchant, 1991]. In addition, *Phaeocystis* is reportedly the principal producer of dimethyl sulfide (DMS) in antarctic waters [Gibson et al., 1990]. Oxidation of this DMS forms sulfate particles which constitute a major source of cloud condensation nuclei (CCN). Bates et al. [1987] and Charlson et al. [1987] propose that the abundance of CCN determines global albedo thereby establishing a mechanism for the regulation of climate by marine biological activity. Gibson et al. [1990] estimate that antarctic *Phaeocystis* may contribute as much as 10% of the total global flux of DMS to the atmosphere.

GRAZING ON *PHAEOCYSTIS*

Although *Phaeocystis* is grazed by herbivores including *Euphausia superba* [Sieburth, 1960; Marchant and Nash, 1986], the effect of grazing on this alga and its food value are equivocal [Verity and Smayda, 1989]. In an investigation on the impact of copepod grazing on a phytoplankton bloom in which *Phaeocystis* comprised about 97% of the biomass and the remainder was mainly diatoms, the diatoms accounted for some 74% of the copepod diet [Claustre et al., 1990]. Only 1.5% of the biomass of *Phaeocystis* was grazed by the copepods, the remainder apparently being lost to the pelagic food web. In addition, Claustre et al. [1990] reported that the low nutritional value of *Phaeocystis* was due to its fatty acid to chlorophyll *a* ratio being much lower than was found in diatoms. This was also the case for amino acids and vitamin C. *Phaeocystis* from antarctic sea ice has been found to have significantly lower concentrations of neutral lipids than diatom assemblages dominated by *Nitzschia* and *Navicula* [Priscu et al., 1990]. Antarctic euphausiids reportedly have a dietary preference for diatoms [Meyer and El-Sayed, 1983; Miller and Hampton, 1989]. We have found that at an antarctic inshore site very little of the carbon attributable to *Phaeocystis* is apparently utilized by metazoa [Davidson and Marchant, 1991] and, as was found by Claustre et al. [1990], most of the carbon was not used *in situ*.

VERTICAL CARBON FLUX IN THE SOUTHERN OCEAN

In addition to the direct contribution of the primary producers, fecal pellets of heterotrophs including protozoa [Nöthig and Bodungen, 1989; Buck et al., 1990] and metazoa, including krill [Wefer et al., 1988], contribute substantially to particulate carbon flux from surface waters of the Southern Ocean. In contrast to the marked seasonality of the sedimentation of primary producers and the feces of grazers, cast exoskeletons of *E. superba* are likely to constitute a major year-round flux of particulate organic carbon from the euphotic zone to deep water or the sediments [Nicol and Stolp, 1989].

UV-ABSORBING COMPOUNDS PRODUCED BY *PHAEOCYSTIS*

We have found that the mucilage of *Phaeocystis* colonies themselves as well as substances secreted into them absorb strongly in the UV region of the spectrum. Axenic cultures of this alga isolated from Prydz Bay, Antarctica, produce extracellular products that absorb strongly at 323 and 271 nm [Marchant et al., 1991]. Absorbance at 271 nm is unlikely to provide protection to the alga additional to that conferred by the attenuation of water [Smith and Baker, 1979]. The motile cells of *Phaeocystis* from Antarctica lack

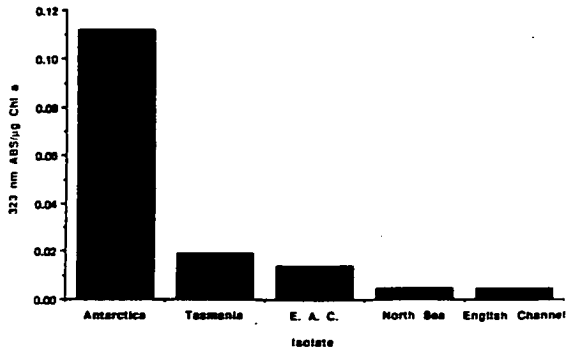


Figure 1. Concentration of 323 nm absorbing pigment per µg chlorophyll *a* from various isolates of *Phaeocystis*. E.A.C. = East Australian Current.

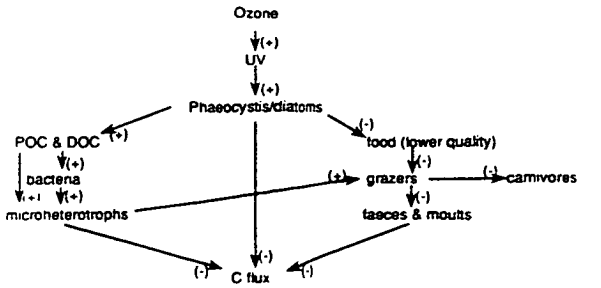


Figure 2. Conceptual diagram of the possible impacts of ozone depletion on Southern Ocean processes. The sign (+ or -) on the arrows indicates the direction of the possible change.

these UV-absorbing substances. Cultures of colonial cells of *Phaeocystis* from the East Australian Current, Tasmanian coastal waters, the North Sea and the English Channel possess these compounds but at substantially lower concentrations than found in antarctic material (Figure 1). The compounds are colorless, water soluble, labile and broken down by bacteria.

These UV-B-absorbing pigments confer a high level of protection to this alga. *Phaeocystis* cultures were exposed to increasing total irradiance using simulated sunlight or increasing UV-B irradiance alone while holding PAR and UV-A constant. Antarctic colonial *Phaeocystis* survived higher irradiances than colonial cells from the East Australian current or motile cells from Antarctica. While *Phaeocystis* has an effective UV-B protective screen, diatom species apparently differ in their level of UV-B screening. Some diatoms apparently lack UV-B-absorbing compounds [Yentsch and Yentsch, 1982]. In those species that do produce these compounds, their concentration is much lower than that found in *Phaeocystis* [J. Raymond, personal communication; A. Davidson, unpublished data]. Thus growth of the colonial stage of *Phaeocystis* rather than diatoms is likely to be favored under elevated levels of UV-B. In *Phaeocystis*-dominated blooms the colonial cell concentration can be very high, reaching 6 x 10^7 cells l^{-1} in antarctic coastal waters [Davidson and Marchant, 1991]. At such concentrations the absorbance in the water column at 323 nm would be about 80% m^{-1} compared with 14% m^{-1} in clear water [Jerlov, 1950] and is thus likely to mitigate UV exposure to co-occurring organisms.

The possible ramifications of increased dominance of *Phaeocystis* at the expense of diatoms in the marginal ice edge zone are indicated in Figure 2. Few data are available to indicate the consequences of such a change in species dominance. If however, as appears to be the case, crustacea selectively graze diatoms in preference to *Phaeocystis*, and diatoms are of greater food value, then there is the possibility that populations of krill and other grazers could be nutrient-limited with a consequent diminution of the food available to higher trophic levels. Reduced availability of more relatively nutritious food may reduce the fecundity of grazers [Verity and Smayda, 1989]. Any diminution in diatom growth is likely to reduce vertical carbon flux. In addition to the reduced flux of feces and molts of grazers that prefer diatoms there would be a decline in the flux of diatoms themselves. The high concentrations of slow-sinking POC and DOC produced by *Phaeocystis* provide substrates for bacteria and microheterotrophs in surface waters. Respiration by these organisms is likely to result in higher concentrations of CO_2 in the photic zone. Further, organisms of the microbial loop are more likely to produce smaller, slower-sinking particles than the feces and molts of grazers and thus constitute a lesser carbon flux than the larger, faster-sinking material.

REFERENCES

Ainley, D. G., W. R. Fraser, C. W. Sullivan, J. J. Torres, T. L. Hopkins, and W. O. Smith, Antarctic mesopelagic micronekton: Evidence from seabirds that pack ice affects community structure, *Science*, *232*, 847–849, 1986.

Bates, T. S., R. J. Charlson, and R. H. Gammon, Evidence for the climatic role of marine sulphur, *Nature*, *329*, 319–321, 1987.

Bodungen, B. v., V. S. Smetacek, M. M. Tilzer, and B. Zeitzschel, Primary production and sedimentation during spring in the Antarctic Peninsula region, *Deep-Sea Res.*, *33*, 177–194, 1986.

Buck, K. R., P. A. Bolt, and D. L. Garrison, Phagotrophy and fecal pellet production by an athecate dinoflagellate in Antarctic sea ice, *Mar. Ecol. Prog. Ser.*, *60*, 75–84, 1990.

Calkins, J., and T. Thordardottir, The ecological significance of solar UV radiation on aquatic organisms, *Nature*, *283*, 563–566, 1980.

Charlson, R. L., J. E. Lovelock, M. O. Andreae, and S. G. Warren, Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate, *Nature*, *326*, 655–661, 1987.

Claustre, H., S. A. Poulet, R. Williams, J.-C. Marty, S. Coombs, F. Ben Mlih, A. M. Hapette, and V. Martin-Jezequel, A biochemical investigation of a *Phaeocystis* sp. bloom in the Irish Sea, *J. Mar. Biol. Ass. U.K.*, *70*, 197–207, 1990.

Davidson, A. T., and H. J. Marchant, Binding of manganese by antarctic *Phaeocystis pouchetii* and the role of bacteria in its release, *Mar. Biol.*, *95*, 481–487, 1987.

Davidson, A. T., and H. J. Marchant, Protist interactions and carbon dynamics of a *Phaeocystis*-dominated bloom at an Antarctic coastal site, *Polar Biol.* (submitted), 1991.

El-Sayed, S. Z., F. C. Stephens, R. R. Bidigare, and M. E. Ondrusek, Effect of ultraviolet radiation on Antarctic marine phytoplankton, in *Antarctic Ecosystems. Ecological Change and Conservation*, edited by K. R. Kerry and G. Hempel, pp. 379-385, Springer-Verlag, Berlin, Heidelberg, 1990.

Fischer, G., D. Fuetterer, R. Gersonde, S. Honjo, D. Ostermann, and G. Wefer, Seasonal variability of particle flux in the Weddell Sea and its relation to ice cover, *Nature*, *335*, 426-428, 1988.

Frederick, J. E., and H. E. Snell, Ultraviolet radiation levels during the antarctic spring, *Science*, *241*, 438-440, 1988.

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

Garrison, D. L., K. R. Buck, and G. A. Fryxell, Algal assemblages in the antarctic pack ice and in ice-edge plankton, *J. Phycol.*, *23*, 564-572, 1987.

Garrison, D. L., and K. R. Buck, The biota of Antarctic pack ice in the Weddell Sea and Antarctic Peninsular regions, *Polar Biol.*, *10*, 211-219, 1989.

Gibson, J. A. E., R. C. Garrick, H. R. Burton, and A. R. McTaggart, Dimethylsulfide and the alga *Phaeocystis pouchetii* in antarctic coastal waters, *Mar. Biol.*, *104*, 339-346, 1989.

Holm-Hansen, O., B. G. Mitchell, and M. Vernet, Ultraviolet radiation in antarctic waters: Effects on rates of primary production, *Antarctic J. U.S.*, *24*, 177-178, 1989.

Jennings, J. C., L. I. Gordon, and D. M. Nelson, Nutrient depletion indicates high primary productivity in the Weddell Sea, *Nature*, *399*, 51-54, 1984.

Jerlov, N. G., Ultra-violet radiation in the sea, *Nature*, *166*, 111-112, 1950.

Jitts, H. R., A. Morel, and Y. Saijo, The relation of oceanic primary production to available photosynthetic irradiance, *Aust. J. Mar. Freshw. Res.*, *27*, 441-454, 1976.

Karentz, D., Ecological considerations of Antarctic ozone depletion, *Antarctic Science*, *3*, 3-11, 1991.

Lorenzen, C. J., Ultraviolet radiation and phytoplankton photosynthesis, *Limnol. Oceanogr.*, *24*, 1117-1120, 1979.

Lubbers, G. W., W. W. C. Gieskes, P. del Castilho, W. Salomons, and J. Bril, Manganese accumulation in the high pH microenvironment of *Phaeocystis* sp. (Haptophyceae) colonies from the North Sea, *Mar. Ecol. Prog. Ser.*, *59*, 285-293, 1990.

Marchant, H. J., and G. V. Nash, Electron microscopy of gut contents and faeces of *Euphausia superba* Dana, *Mem. Natl. Inst. Polar Res. Spec. Issue*, *40*, 167-177, 1986.

Marchant, H. J., A. T. Davidson, and G. J. Kelly, UV-B protecting pigments in the marine alga *Phaeocystis pouchetii* from Antarctica, *Mar. Biol.*, 1991, In press.

Maske, H., Daylight ultraviolet radiation and the photoinhibition of phytoplankton carbon uptake, *J. Plankton Res.*, *6*, 351-357, 1984.

Meyer, M. A., and S. Z. El-Sayed, Grazing of *Euphausia superba* Dana on natural populations, *Polar Biol.*, *1*, 193-197, 1983.

Miller, D. G. M., and I. Hampton, *Biology and Ecology of the Antarctic Krill (Euphausia superba* Dana*): A Review*, BIOMASS Scientific Series No. 9, 166 pp., SCAR & SCOR, Scott Polar Research Institute, Cambridge, 1989.

Nöthig, E.-M., and B. v. Bodungen, Occurrence and vertical flux of faecal pellets of probably protozoan origin in the southeastern Weddell Sea (Antarctica), *Mar. Ecol. Prog. Ser.*, *56*, 281-289, 1989.

Nicol, S., and M. Stolp, Sinking rates of cast exoskeletons of Antarctic krill (*Euphausia superba* Dana) and their role in the vertical flux of particulate matter and fluoride in the Southern Ocean, *Deep-Sea Res.*, *36*, 1753-1762, 1989.

Priscu, J. C., L. R. Priscu, A. C. Palmisano, and C. W. Sullivan, Estimation of neutral lipid levels in Antarctic sea ice microalgae by nile red fluorescence, *Antarctic Science*, *2*, 149-155, 1990.

Roberts, L., Does the ozone hole threaten antarctic life?, *Science*, *244*, 288-289, 1989.

Ross, R. M, and L. B. Quetin, How productive are Antarctic krill, *BioScience*, *36*, 264-269, 1986.

Sakshaug, E., and H. R. Skjoldal, Life at the ice edge, *Ambio*, *18*, 60-67, 1989.

Sieburth, J. McN., Acrylic acid, an "antibiotic" principle in *Phaeocystis* blooms in Antarctic waters, *Science*, *132*, 676-677, 1960.

Smith, R. C., and K. S. Baker, Penetration of UV-B and biologically effective dose-rates in natural waters, *Photochem. Photobiol.*, *29*, 311-323, 1979.

Smith, W. O., Jr., and D. M. Nelson, Importance of ice edge phytoplankton production in the Southern Ocean, *BioScience*, *36*, 251-257, 1986.

Stolarski, R. S., A. J. Krueger, M. R. Schoeberl, R. D. McPeters, P. A. Newman, and J. C. Alpert, Nimbus 7 satellite measurements of the springtime Antarctic ozone decrease, *Nature*, *322*, 808-811, 1986.

Trodahl, H. J., and R. G. Buckley, Ultraviolet levels under sea ice during the antarctic spring, *Science*, *245*, 194-195, 1989.

Truesdale, R. S., and T. B. Kellogg, Ross Sea diatoms: modern assemblage distributions and their relationship to ecologic, oceanographic and sedimentary conditions, *Mar. Micropaleontol.*, *4*, 13-31, 1979.

Verity, P. G., and T. J. Smayda, Nutritional value of *Phaeocystis pouchetii* (Prymnesiophyceae) and other phytoplankton for *Acartia* spp. (Copepoda): ingestion, egg production, and growth of nauplii, *Mar. Biol.*, *100*, 161-171, 1989.

Wefer, G., G. Fischer, D. Fuetterer, and R. Gersonde, Seasonal particle flux in the Bransfield Strait, Antarctica, *Deep-Sea Res.*, *35*, 891-898, 1988.

Wood, W. F., Effect of solar ultra-violet radiation in the kelp *Ecklonia radiata*, *Mar. Biol.*, *96*, 143-150, 1987.

Wood, W. F., Photoadaptive responses of the tropical red alga *Eucheuma striatum* Schmitz (Gigartinales) to ultraviolet radiation, *Aquatic Bot.*, *33*, 41-51, 1989.

Worrest, R. C., Impact of solar ultraviolet-B radiation (290-320 nm) upon marine microalgae, *Physiol. Plant.*, *58*, 428-434, 1983.

Yentsch, C. S., and C. M. Yentsch, The attenuation of light by marine phytoplankton with special reference to the absorption of near-UV radiation, in *The Role of Solar Ultraviolet Radiation in Marine Ecosystems*, edited by A. J. Calkins, pp. 691-706, Plenum, New York, 1982.