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Biogeochemistry and phytoplankton dynamics in the Ross Sea, Antarctica

Amy Rebecca Shields
College of William and Mary - Virginia Institute of Marine Science

BIOGEOCHEMISTRY AND PHYTOPLANKTON DYNAMICS IN THE ROSS SEA, ANTARCTICA

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Amy Rebecca Shields

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APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

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Amy R. Shields

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Walker O. Smith, Jr., Ph.D.
Major Advisor

Elizabeth A. Canuel, Ph.D.

Deborah K. Steinberg, Ph.D.

Kam W. Tang, Ph.D.

David A. Caron, Ph.D.
University of Southern California
Los Angeles, CA

DEDICATION

I dedicate this dissertation to the Barr and Shields families, for their compassion, patience, and guidance that made this all possible.
To my niece and nephew, Ella and Blake Lucchi, may you grow to love Antarctica, respect the planet, and explore the world through science.

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ABSTRACT

The Ross Sea, Antarctica seasonal phytoplankton bloom is one of the largest in the Southern Ocean. This project focuses on the biological pump, which removes carbon from the surface ocean to the deep ocean through the settling of particulate organic matter, the advection of dissolved organic carbon, and active flux due to vertical migration of zooplankton. The objective of this study was to focus on three interrelated components of the biological pump including sedimentation, photosynthetic rates and grazing. The study was conducted in coordination with the Interannual Variability in the Antarctic-Ross Sea program, which covered the time period between 2001-2005. Simple, one-dimensional budgets were made using *in situ* nitrogen and silica concentrations and published climatologies. There was significant interannual and seasonal variability in phytoplankton bloom composition and concentrations of organic matter. During February 2004, a large secondary bloom of diatoms occurred, and nitrate removal was 8-fold higher than during other years in the study period. Principal components analysis was utilized to examine patterns in the large data set. Through visualization of the loadings and scores of the principal components, the primary controls of the concentrations of biomass and organic matter were seasonality, phytoplankton community composition and temperature, which explained 68.1 % of the variance of the data set. There was also a significant negative relationship between the percent abundance of *Phaeocystis antarctica*, a dominant phytoplankton group, and temperature. Vertical flux measurements at 200 m using sediment traps showed that fecal pellet carbon during certain periods (February 2004, 2005) represents a large percentage of the total carbon flux from the surface, which suggests that mesozooplankton were actively grazing and packaging phytoplankton into sinking pellets. Photosynthesis/Irradiance measurements were the first to show that colonial *P. antarctica* may have higher growth rates early in the growing season, which may be one reason why large *P. antarctica* blooms occur earlier that diatoms. Lastly, preliminary results utilizing a novel fluorescently labeled algae technique showed colonial *P. antarctica* can be grazed by zooplankton and enter the food web before sedimentation.

BIOGEOCHEMISTRY AND PHYTOPLANKTON DYNAMICS IN THE ROSS SEA, ANTARCTICA

Project Introduction

The Biological Pump

Oceanic primary production accounts for nearly 50% of the total global primary production. Phytoplankton in the open ocean account for ca. 80% of this marine production (Martin et al., 1987). These primary producers are part of the biological pump which, is the principal biological regulator of ocean-atmosphere carbon cycling. The biological pump removes carbon from the surface ocean to the deep ocean through the settling of particulate organic matter, the physical mixing of dissolved organic carbon, and active flux due to diel vertical migration (Longhurst and Harrison, 1989; Ducklow et al., 2001). The organic matter in the surface waters either enters the microbial loop and is remineralized in the surface or it sinks out of the surface waters through active grazing by zooplankton (fecal pellets or vertical migration) or sedimentation. The biological pump includes three interrelated processes that will be the focus of this dissertation: primary production, export, and the role of grazers in the acceleration of carbon flux to depth.

Nutrient sources such as nitrate and ammonium support this organic matter production. Primary production supported by regenerated sources of nitrogen such as ammonium (NH_4^+) is considered to be regenerated production, while new production is the total primary production that is supported by nutrient sources that came from deeper water sources of nitrate through mixing or upwelling (Dugdale and Goering, 1967). New production represents the carbon that is available for export and often is dominated by larger phytoplankton which, are more actively grazed by larger consumers and have higher sedimentation rates.

The Ross Sea, Antarctica

The Ross Embayment stretches from 165° E to 155°W longitude and is located in the Pacific sector of the Antarctic continent. The Ross Sea is characterized by having high levels of biogenic production (Nelson and Smith, 1986), confined boundaries, and is located near McMurdo Station, a major Antarctic facility (DeMaster et al., 1992). The Ross Sea polynya (an area of reduced ice cover surrounded by ice) has predictable phytoplankton blooms due to the physical properties of annual sea ice retreat and water stratification (Arrigo et al., 1999), with a seasonal chlorophyll maxima of over 10-15 μg L^{-1} (Smith et al., 2000). Ice is advected away from the Ross Sea Ice Shelf by katabatic winds, exposing surface waters and driving new ice formation (Smith and Gordon, 1997). Abiotic properties such as light, temperature, and dissolved nutrients control the primary production and regulate phytoplankton growth. This seasonal phytoplankton bloom is one of the largest blooms in the Southern Ocean, with spatial coverage of ca. 187,000 km^2 (Smith and Nelson, 1985; Comiso et al., 1993; Sullivan et al., 1993). Although interannual variability occurs in this region, seasonal effects have been suggested as being greater than interannual production cycles (Smith et al., 2000). Blooms in the southern Ross Sea are dominated by two taxa that are spatially distinct (Smith et al., 1996): *Fragilariopsis curta*, a pennate diatom, dominating the coastal region and *Phaeocystis antarctica*, a colonial prymnesiophyte, dominating the south central region (Smith and Nelson, 1985; Arrigo et al., 1999). The reason for this gradient of phytoplankton dominance is still unclear, but researchers have speculated that subnanomolar concentrations of iron in the Ross Sea might be limiting phytoplankton growth (deBaar et al., 1995).

Phytoplankton ecology and biogeochemistry

Phytoplankton community composition in the Ross Sea has direct influence on vertical flux and element removal ratios (DeMaster et al., 1992; Smith and Dunbar, 1998; Arrigo et al., 1999; Smith et al., 2006). During the past 15 years, the biogeochemistry of the Ross Sea has been intensively investigated by several large programs (e.g., Smith and Anderson, 2003), and the details of the carbon, nitrogen and silica budgets are as well known as at any location in the Antarctic (Nelson et al., 1996; Sweeney et al., 2000a; Arrigo et al., 2003). In addition, satellite observations have provided both continuous observations on ice distribution and concentration, as well as estimates of phytoplankton pigment concentrations (Comiso et al., 1993; Arrigo and McLain, 1994; Arrigo et al., 2003).

Estimates of net community production based on carbon budgets were first made by Bates et al. (1996), and others have made similar estimates or used nitrogen to estimate seasonal production and export (Smith and Asper, 2000, 2001; Sweeney et al., 2000a,b). These annual estimates of biomass production have resolved the magnitude of the biogeochemical pathways during a single year. However, direct observations designed to assess interannual variability of net community production are unavailable to date.

The Southern Ocean is the largest high nutrient, low chlorophyll region in the world, with average nitrate surface concentrations of 25 μM (Fitzwater et al., 2000). Therefore, the vertical input of nitrogen is not required to sustain growth (Smith and Dunbar, 1998). In order to completely understand biogeochemical cycling in the Ross Sea, a nitrogen budget for the surface layer is important. But, due to difficulties of lateral

advection, use of discrete measurements, and elemental transformations, it is difficult to collect data on appropriate space and time scales (Smith and Asper, 2000). However, nitrification and denitrification are negligible during the summer in the Southern Ocean, so the nitrogen budget is somewhat simplified when compared to other marine systems. Therefore, changes in the pools of nitrate can be simplified to changes in particulate nitrogen (PN), nitrite (NO_2^-), ammonium (NH_4^+), dissolved organic nitrogen (DON), and flux of particulate nitrogen to depth (F_{PN}) (Smith and Asper, 2000):

$$\Delta NO_3^- = \Delta PN + \Delta NO_2^- + \Delta NH_4^+ + \Delta DON + F_{PN} \quad (1)$$

Smith and Asper (2000) found that nitrite concentrations were low (mean= 0.037 $\pm$ 0.025 μM; n=1407). Ammonium concentrations before the bloom were only ca. 5% of the nitrogen pool. Using the simplified equation for the changes in pools of nitrogen in the Ross Sea, Smith and Asper (2000) found that nitrate uptake by phytoplankton increased as the spring bloom developed and increased through early January. Previous measurements of nitrate in the Ross Sea in 1990 and 1992 in January and February ranged from 15.4 μM to 26.4 μM (Smith et al., 1996). These results are also supported by other studies in which phytoplankton blooms resulted in removal of nutrients and carbon dioxide in the surface waters (Arrigo and McClain, 1994; Sweeney et al., 2000a). The role of DON production was minor with an increase of 0.67 μM over 75 days (Smith and Asper, 2000) which, is also supported by net changes in dissolved organic carbon concentrations (Smith et al., 1998). Species composition did not influence nitrogen inventories, but it was noted that species specific nitrogen uptake became less important since analysis was completed over seasonal time scales (Smith and Asper, 2000).

Asper and Smith (1999) have found that the export of material from the euphotic zone was coupled with the surface layer production and biomass. In addition, as the bloom of *Phaeocystis antarctica* reaches its maximum biomass, aggregates form. Grazing is thought to have little influence on vertical flux in the Ross Sea when colonies of *Phaeocystis* are present. Grazing on colonies and its effect on sinking rates due to fecal pellet production is complex and is affected by animal physiology and microbial colonization of *P. antarctica* colonies (Bautista et al., 1992). Since primary productivity is often dominated by *P. antarctica* (Smith and Gordon, 1997), the export flux usually consists of rapidly sinking colonies and aggregates. Although vertical flux rates increased in summer, vertical flux rate measurements by Asper and Smith (1999) found that there was a temporal uncoupling between phytoplankton productivity and biomass with export.

Diatoms

Globally, diatoms contribute disproportionately to organic matter export compared to primary productivity, primarily due to the fact that diatoms dominate in nutrient-rich systems where export is high and because of their size and mineral ballasting (Buessler, 1998; Armstrong et al., 2002). In addition, their fate is influenced primarily to be grazed by meso- and macrozooplankton which, in turn package the diatoms into large fecal pellets (Nelson et al., 1996). Therefore, diatoms are a significant component of the "biological pump", in which CO_2 is fixed, grazed, and transported to the deep ocean. During intense diatom blooms in the Ross Sea, the mean silica production rate can reach 38 mmol Si m^{-2} d^{-1} (DeMaster et al., 1992). DeMaster et al. (1992) used a silica budget for the water/sediment column of the Ross Sea, and only

5.8% of the opal produced annually was buried long-term in the sea-bed. The Ross Sea, therefore, is lower than the rest of the Southern Ocean in overall preservation efficiency of biogenic silica. Nelson et al. (1996) noted that this was primarily due to the depth of the Ross Sea being inadequate for breeding populations of most euphausiids.

Phaeocystis antarctica

Large blooms of the genus *Phaeocystis* occur worldwide, including in the Ross Sea, North Sea, Greenland Sea, Arabian Sea, and the Barents Sea (Lancelot et al., 1998). Colony development is sustained by new sources of nitrate (deep convection) or anthropogenic sources (coastal areas), and there is a positive relationship between maximum chlorophyll *a* concentrations reached by colonies and nitrate reduction (Lancelot et al., 1998). Blooms of the colonial prymnesiophyte, *Phaeocystis antarctica*, are known to dominate the central region of the Ross Sea polynya (Smith and Gordon, 1997). Deep mixed layers in the Ross Sea have been suggested to sustain *P. antarctica* growth relative to diatoms (Arrigo et al., 1999), reflecting their ability to have greater photosynthetic rates at lower irradiances (Moisan and Mitchell, 1999). However, Smith and Asper (2001) found that mixed layer depths were not significantly different between stations dominated by diatoms and *Phaeocystis* dominated.

Three species of *Phaeocystis*, including *P. antarctica*, exist mainly as single, flagellated cells and as non- flagellated cells in colonies (Lancelot et al., 1998; Rousseau et al., 1994). *Phaeocystis* has a complicated and poorly understood life cycle. It is known that there are several life stages in which a motile cell with flagella can form hollow, spherical colonies with an effective spherical diameter greater than 1 mm, with active division of the cells within the matrix (Mathot et al., 2000). During colony development,

a clear and viscous sticky mucoid sheath is formed. The mucoid sheath functions as an organic matrix which is the basis of the colony (Hamm, 2000). Although little is known about what environmental factors control colony formation and release of solitary cells from colonies, inorganic nutrient concentrations have been speculated to influence the form of *Phaeocystis* (Verity et al., 1988).

Photosynthesis/Irradiance experiments

Smith et al. (2003b) suggest that grazing might not be the only factor regulating the abundance of solitary and colonial cells of *P. antarctica*. Physiological differences might allow them to respond to varying environmental conditions. Solitary cells are significantly smaller than colony cells, with single cells measuring about 3.1 $\pm$0.6 μm and colonial cells measuring 5.1 $\pm$ 1.1 μm (Mathot et al., 2000). Lancelot and Mathot (1985) also found that during the colonial stages of other species of *Phaeocystis* (*P. pouchetii*), that the mucous envelope acts as an intracellular reserve for the cells during the dark period. Therefore, part of the carbon fixed by *P. pouchetii* colonies is allocated to extracellular carbon production (Lancelot and Mathot, 1985). *Phaeocystis* also acclimates to abrupt irradiance changes by xanthophyll cycling (Moissan et al. 1998), while increasing the amount of pigment per cell to adapt to low light levels. This adaptation makes *Phaeocystis* able to dominate polar regions due to "bottom up" controls (Moisan and Mitchell, 1991).

Photosynthesis and its relationship with irradiance is modeled through numerous equations. The most common is the Platt et al. (1980) equation which, follows the empirical relationship:

$$P^B = P^B_s [1 - \exp(-\alpha E / P^B_s)] \exp(-\beta E / P^B_s) \qquad (2)$$

with the carbon fixation rate, P, normalized to chlorophyll (B). Three primary parameters of photosynthesis are the maximum fixation rate without photoinhibition, P^B_S [(mg Chl a)$^{-1}$ h^{-1}], α [mg C (mg Chl a)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})], the initial slope of the light saturation curve, and β, which, is the amount of photoinhibition (Platt et al 1980). The photosynthetically active radiation or E (μmol m^{-2} s^{-1}), is the independent variable. Two derived parameters from these variables are P^B_m (maximum carbon fixation rate) and E_k (adaptation parameter).

They are given by:

$$P^B_m = P^B_S((\alpha/(\alpha+\beta)) * (\beta/(\alpha+\beta))^{\beta/\alpha} \quad (3)$$

$$E_k = P^B_m / \alpha. \quad (4)$$

The photosynthesis/irradiance curve describes the non-linear relationship photosynthetic rates have with irradiance. There are three major regions of the resultant curve in which photosynthesis responds to irradiance. In the first region (α), photosynthetic rates are linear with irradiance and the absorption of photons is slower than the cells ability or capacity rate of steady-state electron transport from water to carbon dioxide. When irradiance increases, irradiance and photosynthetic rates become non-linear and rise to saturation. This is because photon absorption being much greater than the steady-state electron transport from water to carbon dioxide in the second region, P^B_m. The third region (β) is a region of reduced photosynthetic rates due to photoinhibition (Sakshaug et al., 1997).

Photosynthetic parameters of Ross Sea phytoplankton increased as the growing season progressed, thereby indicating a temporal acclimation to changing irradiances (van Hilst and Smith, 2002). Parameters (α, , β, P^B_m, and E_k) of both diatom dominated

and *Phaeocystis antarctica*-dominated assemblages were compared, and it was concluded that phytoplankton community composition did not play a major role in regulating photosynthetic performance. At lower irradiances α was higher, while values for E_k were lower in both field and lab studies cultures dominated by diatoms and *Phaeocystis*. Antarctic diatoms and *P. antarctica* are well adapted to low irradiances (van Hilst and Smith, 2002; Brightman and Smith, 1989). It is clear from these previous results that numerous environmental processes control phytoplankton dynamics in the Ross Sea including grazing, spatial and temporal variations, and micronutrient limitation. Polar environments exhibit variable chemical, physical, and biological processes, making it unlikely that a single process controls the dynamics of a phytoplankton population (van Hilst and Smith, 2002).

Objectives

This study was designed to describe the interannual variability in phytoplankton bloom dynamics and their primary controls including sedimentation, photosynthetic rates, and grazing. Differences in biogeochemical cycling between austral spring and summer in the Ross Sea have been explored in great detail, but variations between years have not. By combining experiments on biological processes such as photosynthesis/irradiance experiments and grazing experiments with the investigation of Interannual Variability in the Antarctic-Ross Sea (IVARS) program data, this dissertation describes how physical, biological, and chemical properties vary over time. These variations will not only influence nutrient budgets, but also the export of organic matter. The following hypotheses are addressed:

1. Interannual variations in net community production, nutrient uptake, and export will occur between years as a function of the magnitude and composition of the bloom. A series of cruises was completed in the southern Ross Sea during 2001-2002, 2003-2004, and 2004–2005. Nutrient data were collected from within transects of largely ice-free regions, and simple, one-dimensional nutrient budgets were made using nitrogen and silica concentrations. From these budgets diatom and total phytoplankton net production were estimated and compared to the known distribution of phytoplankton. Finally, export was calculated by a comparison of the particulate matter distribution in the upper 200 m with that of nutrient disappearance in order to contrast this estimate with those of other years determined by either nutrient budgets or direct collections of particle flux.

2. **Hydrographic parameters including temperature will drive phytoplankton biomass, assemblage composition, and nutrient uptake in the early growing season of December 2001, 2003 and 2004.** Principal components analysis was performed on three years of the data set comprised of austral spring and summer data including dissolved nutrients (N,P,Si), size fractionated chlorophyll (>20, >0.7, and <20 μm), depth of sample (0, 20, and 50 m), biogenic silica, particulate organic carbon, particulate nitrogen, temperature, and phytoplankton pigments. Interpretation of these results helps explain the primary factors affecting phytoplankton bloom formation in austral spring.

3. **Phytoplankton bloom composition (diatoms or *Phaeocystis*) will affect the export of carbon, biogenic silica, and nitrogen out of the euphotic zone in the Ross Sea.** Two sediment trap moorings representing historically different biogeochemical regimes (diatoms and *Phaeocystis)* were deployed during 2003-2004 and 2004-2005. Interannual

variations in total mass flux, biogenic silica, and particulate organic carbon were measured. The morphology of fecal pellets and the role they play in sediment flux will also be discussed.

4. **The solitary form of *P. antarctica* will have higher rates of carbon fixation than the colonial form due to the former's ability to thrive during resource and light limitation.** The relative photosynthetic potential of solitary and colonial *P. antarctica* cells and mixed phytoplankton assemblages in December of 2001, 2003, and 2004 is assessed in order to determine the magnitude of interannual variability of photosynthesis in the Ross Sea. Further analysis of ancillary data provides details on how growth stage of the bloom may affect colonial *P. antarctica* photosynthetic rates. *P. antarctica* colonies dominate primary production in the Ross Sea and are critical to biogeochemical cycling.

5. **Microzooplankton ingestion rates of solitary and colonial *P. antarctica* cells will not significantly differ due to the ability of microzooplankton to graze the colonial matrix.** The main purpose of this chapter was determine the relative ingestion rates of solitary and colonial *P. antarctica*, and the drawbacks in using this method in the field will also be discussed. A novel dual staining method was developed in order to determine the feeding preference of a ciliate, *Euplotes* from the Ross Sea. A pilot experiment was performed in order to address the ability of *Euplotes* to ingest colonial *P. antarctica*. Assessments of microzooplankton grazing of colonial *P. antarctica* are important to the biological pump as blooms of *P. antarctica* may enter the microbial food web in the Ross Sea as opposed to direct sedimentation of colonies or single cells.

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Section I. Interannual variations in nutrients, net community production and biogeochemical cycles in the Ross Sea, Antarctica*

(Modified with excerpts from Smith et al. 2006)

* This section is a modification of a manuscript that included two years of data presented in:
Smith, W.O., Jr., Shields, A.R., Peloquin, J.A., Catalano, G., Tozzi, S., Dinniman, M.S., and V.A. Asper. 2006. Interannual variations in nutrients, net community production and biogeochemical cycles in the Ross Sea. Deep Sea Research II 53: 815-833.

ABSTRACT

During the past 15 years, investigations of the biogeochemistry of the Ross Sea have demonstrated that the Ross Sea as a whole is the most productive region in the entire Southern Ocean. The Ross Sea, like the rest of the Antarctic, is characterized by extreme seasonal changes in physical, chemical and biological processes. This section reports the results of a series of cruises completed in the southern Ross Sea during 2001-2002, 2003-2004, and 2004–2005. Nutrient data were collected from within transects of largely ice-free regions, and simple, one-dimensional nutrient budgets were constructed using nitrogen and silica concentrations. I hypothesized that variations in nutrient dynamics and production would occur between years not only in terms of both magnitude and composition of the bloom, but also in the controlling mechanisms. Nitrate concentrations during the three years varied substantially, with resultant February nitrate concentrations in the surface layer in February 2004 (16.8 ± 2.1) significantly lower than February 2001 and 2005 (22.0 ± 1.7, 2001; 19.7 ± 1.9, 2005). The mean concentration of silicic acid in February 2004 (45.8 ± 5.0) was also significantly less than in 2002 and 2005 (65.4 ± 2.6, 2002; 66.2 ± 10.4, 2005). This suggests that removal of silicic acid in 2001–2002 in spring was reduced relative to the long-term mean calculated from the historical climatology. The variations in relative nutrient uptake can be best explained by variations in phytoplankton species composition effects, but also are influenced by variations in Si/N uptake ratios. Export of biogenic material from the surface layer (200 m), calculated by mass balance, also showed substantial differences among years. Annual nitrogen disappearance was 4-fold greater in 2003–2004 than for the entire season in 2001–2002, although in spring the export was only 2.1-fold greater. Water mass

intrusions may have caused the large, secondary diatom bloom in 2003-2004. It is impossible to know if the large diatom blooms observed in February 2004 will be a consistent feature in the future, but we do know that these diatom accumulations were much greater than was observed in studies conducted during the 1990s. Because phytoplankton species composition plays such a critical role in both food web structure and biogeochemical cycles, knowledge of the primary controls of bloom formation is critical to our understanding of energetic and elemental cycles of the Ross Sea.

INTRODUCTION

Biogeochemistry of the Ross Sea

During the past 15 years, the biogeochemistry of the Ross Sea has been intensively investigated by several large programs (e.g., Smith and Anderson, 2003), and the details of the carbon, nitrogen and silica budgets are as well known as any location in the Antarctic (Nelson et al., 1996; Sweeney et al., 2000a; Arrigo et al., 2002). In addition, satellite observations have provided both continuous observations on ice distribution and concentration, as well as estimates of phytoplankton pigment concentrations (Comiso et al., 1993; Arrigo and McLain, 1994; Arrigo et al., 2003). Direct estimates of primary production (Smith and Gordon, 1997; Saggiomo et al., 1998) and of vertical fluxes of biogenic material (Dunbar et al., 1998; Asper and Smith, 1999; Collier et al., 2000; Accornero and Gowing, 2003) also have been made. These biomass and production measurements have demonstrated that the Ross Sea as a whole is the most productive region in the entire Southern Ocean. Annual productivity estimates exceed 200 g C m^{-2}, and while not exceptionally great relative to the entire ocean, they are high when the length of the growing season (ca. 120 days at most) is considered. Estimates of net community production were first made by Bates et al. (1996) based on carbon budgets, and others have made similar estimates or used nitrogen to estimate seasonal production and export (Smith and Asper, 2000, 2001; Sweeney et al., 2000a,b). These annual estimates of biomass production have resolved the magnitude of the biogeochemical pathways during a single year. However, to date direct observations designed to assess interannual variability of net community production are unavailable.

Ross Sea Hydrology

The physical forcing of the Ross Sea structures the biogeochemical cycles of the region. The generalized current pattern consists of a strong current that follows the contours of the shelf break; on the shelf, currents flow along the coast of Victoria Land, and also form gyres that roughly follow the bathymetry (Jacobs and Giulivi, 1998; Dinniman et al., 2003). Cross-shelf exchanges with the Antarctic Circumpolar Current also occur at bathymetric discontinuities and result in the movement of Modified Circumpolar Deep Water (MCDW) onto the shelf. The MCDW waters are relatively warm and likely play a role in the maintenance of the Ross Sea polynya; however, the strength and duration of these intrusions are unknown. Recently large icebergs have calved off the Ross Ice Shelf and grounded in various locations on the continental shelf. In addition to altering the surface advection of ice (Arrigo et al., 2002), icebergs also have modified the currents for relatively long periods (years) (Dinniman et al., in press). The changes in currents, as well as the observed variations in ice cover (Kwok and Comiso, 2002) have the potential for creating marked variations in biological processes on a variety of time scales.

The Ross Sea, like the rest of the Antarctic, is characterized by extreme seasonal changes in physical, chemical and biological processes. Smith et al. (2000) showed distinct seasonal patterns in phytoplankton photosynthesis, production, growth and biomass, and that each variable was temporally uncoupled from the others. These authors concluded that seasonal patterns were significantly greater than interannual trends. Furthermore, phytoplankton growth appeared to be controlled by irradiance in spring and micronutrient concentrations in summer, whereas loss processes largely controlled

biomass accumulation. No determination of the effects of phytoplankton species composition to losses or vertical flux was made.

Nutrient and pigment data for the Ross Sea have been compiled, and a monthly climatology of nitrate, silicic acid and chlorophyll concentrations generated using objective techniques (Smith et al., 2003a). This climatology confirmed results from individual cruises suggesting that the major portion of nitrate uptake occurred during austral spring, whereas most of the silicic acid uptake occurred in summer (Nelson et al., 1996; Smith and Gordon, 1997). Because all members of the phytoplankton community influence nitrate concentrations, but only diatoms modify silicic acid levels, the seasonal patterns of net community production can be roughly partitioned into major functional groups (that is, diatoms and *Phaeocystis antarctica*). It appears that the growth of *P. antarctica* in the southern Ross Sea occurs in large part in the central portion of the Ross Sea polynya, beginning in November and reaching a biomass maximumin late December. In contrast, diatom growth occurs both in the eastern and western sections near ice edges and is maximal in January and early February. Unfortunately, few seasonal data exist to adequately resolve the spatial and seasonal patterns suggested by the climatology.

Objectives

This paper reports the results of a series of cruises completed in the southern Ross Sea during 2001-2002, 2003-2004, and 2004–2005. For data collected during 2002-2003, see Smith et al. (2006). Nutrient data were collected from within transects of largely ice-free regions, and simple, one-dimensional nutrient budgets were made using nitrogen and silica concentrations. From these budgets diatom and total phytoplankton net production were estimated and compared to the known distribution of phytoplankton. Finally, export

was calculated by a comparison of the particulate matter distribution in the upper 200 m with that of nutrient disappearance, in order to contrast this export estimate with those of other years determined by either nutrient budgets or direct collections of particle flux. I hypothesize that variations occur among years, not only in terms of both magnitude and composition of the bloom, but also in the controlling mechanisms.

METHODS

Sample collection

Water samples were collected from the southern Ross Sea during three field seasons (2001–2002, 2003-2004, and 2005-2006) in late spring (mid- to late December) and late summer (mid-February) using the USCGC *Polar Star* and RVIB *N.B. Palmer*. Station locations were part of a transect that was roughly parallel to the Ross Ice Shelf, but were largely chosen to sample ice-free waters (Figure 1; Smith et al., 2006). Samples were collected using 10-L Niskin bottles mounted on a rosette frame which, also housed a SeaBird 911+ CTD and Chelsea fluorometer to collect continuous profiles of temperature, salinity, and density during water sampling from discrete depths. Detailed methodology of sampling depths and processing with a total of 12 depths sampled to 200 m is provided in Smith et al. (2006). Temperature, salinity and derived density data were binned into 1-m intervals, and mixed layer depths derived from the vertical distribution of density (Z_{mix} was defined as the depth where σ_T changed by 0.1 units from a stable, surface value; Smith et al., 2000). Complete hydrographic data are available at http://www.vims.edu/bio/ivars/. Detailed information about ice concentration data is available at http://www.nsidc.org and Smith et al. (2006).

Samples were collected for nutrients (nitrate+nitrite, silicic acid, phosphate), chlorophyll (size fractionated: >5 μm, >20 μm, and total), pigments, particulate organic carbon (POC), particulate nitrogen (PN), and biogenic silica (BSi). Samples (60 mL) for nutrients with elevated chlorophyll levels (those with approximately 1.0 $\mu g\ l^{-1}$ chlorophyll *a* or more) were filtered through Gelman Acrodiscs (5.0 μm) and frozen at -80 °C for later analysis using standard, automated techniques. Chlorophyll samples were filtered through either polycarbonate (20 or 5 μm; Poretics) or Whatman GF/F filters, placed in 7 mL 90% acetone, and sonicated for 15 min. After extraction for at least another 15 min on ice in darkness, the filter was removed and the sample read on a Turner Designs Model AU fluorometer before and after acidification. The fluorometer was calibrated before and after the cruise using commercially purified chlorophyll (Sigma) and checked using high-performance liquid chromatography. POC and PN were determined by filtering known volumes of water through precombusted GF/F filters, rinsed with ca. 5 mL 0.01N HCl in seawater, and drying the filters in combusted glass vials at 60° C. Blanks were filters placed under the deep-water sample's filter, and these filters were processed identically to the other samples. All filters were analyzed using a Carlo–Erba Model 254 elemental analyzer (Smith et al., 1996). Samples for biogenic silica were filtered through 0.6-μm Poretics polycarbonate filters, dried in plastic Petri dishes at 60 °C, and returned to the laboratory. The samples then were digested in NaOH at 100 °C for 40 min, neutralized and analyzed colorimetrically for reactive silicate on a dual-beam spectrophotometer (Brzezinski and Nelson, 1989). Contributions of lithogenic Si to total BSi are small in the Ross Sea (1%; Nelson, unpublished) and were ignored.

Samples for pigment analysis by HPLC were collected and analysis and data processing are described in detail in Smith et al. 2006.

Data analysis

The basic method we used to assess net community production is similar to that used by Bates et al. (1998), Smith and Asper (2000) and Sweeney et al. (2000a). Because two, discrete periods were sampled, the temporal dynamics of nutrient uptake could be more completely assessed and related more closely to assemblage composition. Specifically, seasonal nitrate uptake (by removal from winter values) was related to net community production at each sampling by

$$\Delta\,(NO_3^-) = \int_0^z (NO_3^-)_{\text{winter}}\,\partial z - \int_0^z (NO_3^-)_{\text{min}}\,\partial z$$

where z is depth, and the subscripts min and winter refer to the observed nitrate concentrations in the water column and the winter nutrient concentration, respectively. The nitrogen units were converted to carbon units using the measured molar C/N ratio of particulate matter; all integrations were from 0 to 200 m. An integration depth of 200 m was chosen because this is below the depth of nutrient removal during austral summer, and flux to greater depths can be considered to be "lost" from the surface layer on at least seasonal time scales. Similarly, the production of diatoms was estimated using

$$\Delta\,Si(OH)_4^- = \int_0^z (Si(OH)_4^-)_{\text{winter}}\,\partial z - \int_0^z (Si(OH)_4^-)_{\text{min}}\,\partial z$$

and converted to carbon units using the molar C/Si ratio (1.61) measured by Nelson and Smith (1986) for blooms overwhelmingly dominated by diatoms. There are no data to suggest that winter values of silicic acid and nitrate in the Ross Sea change on decadal scales, and vertical mixing during winter makes nutrient concentrations uniform throughout the water column. Hence, nitrate and silicic acid concentrations can be reliably predicted from the AESOPS and RSP2 data (e.g., nitrate values are 31.0 μM when normalized to S 35 psu, and silicic acid values are 80 μM; Smith and Asper, 2000; http://usjgofs.whoi.edu/jg/dir/jgofs/southern/). Nitrate potentially can be remineralized within the growing season via nitrification, but this process is extremely slow at the low temperatures of the Ross Sea and was ignored (Karl et al., 1996). Integrated NH_4^+ concentrations are less than 5% of the total inorganic nitrogen concentrations at all times, and are ignored for these calculations. The particulate nitrogen and silicon distributions were measured, and integrated values compared to the nutrient removal values; the difference was taken to be an estimate of export, although we realize that for nitrogen some portion of the material may have entered the dissolved organic nitrogen (DON) pool. All assumptions included those about low rates of regenerated production used in the calculations are discussed in Smith and Asper (2000). Phosphate was not treated in this analysis due to a more limited particulate phosphorus data set.

RESULTS

Hydrographic data

Ice coverage varied both in time and space during our study period (Smith et al., 2006; Figure 2). During 2001-2002 ice distributions were similar to the mean condition

for the region. In 2003-2004, ice concentrations were higher than the long term mean. Ice coverage in 2004-2005 was similar to 2001-2002. Sea surface temperature was significantly higher in December 2004 than December of other years (Table 1, ANOVA, DF=28, F=15.10, $p<0.0001$). Surface salinity was significantly lower in December 2003 (Table 1, ANOVA, DF=28,F=41.60, $p<0.0001$). There was no significant difference in mixed layer depth, which may be due to the variability of mixed layer depths between stations. Surface σ_T was also significantly lower in December 2003 (Table 1, ANOVA DF=25, F=44.86, $p<0.0001$).

Nutrient data

December 2004 had the lowest mean concentration of nitrate at the surface (16.5 ± 3.0 μm nitrate) (Table 2, ANOVA, DF=25, F=10.93, $p<0.0001$). Nitrate distributions suggest large differences in phytoplankton removal occurred between all three years. In 2001-2002 and 2004-2005 a majority of nitrate removal occurred from the start of the growing season through late summer. Nitrate removal during January and February of 2005 was substantially reduced (Figure 3a-3f).

Silicic acid profiles were more variable than nitrate profiles, with large removals of silicic acid in December 2003 and 2004, when compared to 2001 (Fig 4a-f). Removal of silicic acid during January and February of 2004 was significantly higher than the other years with a mean surface value of 45.8 ± 5.0 μM due to a secondary diatom bloom that year (Table 2, ANOVA, log transformed, DF=20, F=15.88, $p<0.0001$).

There were between-year differences in phosphate concentrations in December (Table 2, ANOVA, DF=25, F=52.0, $p<0.0001$). One unexpected trend in 2005 was an increase in all dissolved nutrients (N, P, Si) during February 2005 when compared to

December of that growing season. This could relate to physical processes adding nitrate into the surface from other sources (ice melt or intrusions of deep water) coupled with reduced biological uptake. Water mass intrusions during December 2004 were extremely high and may have provided an additional pulse of nutrients and are discussed further in Section II. The water mass mentioned by Peloquin and Smith (2006) exhibited different temperature/salinity signals during December 2003 than in December 2004. Initial analysis of these physical properties and its relationship to the phytoplankton assemblage are currently being performed (Peloquin, unpublished).

Pigments

Surface chlorophyll concentrations were similar in December 2001, 2003, and 2004 (Table 2, Figure 5a-f). As expected, chlorophyll concentrations increased with uptake of nitrate. However, during February 2004, chlorophyll concentrations were high compared to February 2002 and 2005 with a mean chlorophyll value of 11.1 ± 4.3 $\mu g\ L^{-1}$. Smith et al. (2006) did further analysis on the HPLC measurements during all three years discussed, and they can be found at http://www.vims.edu/bio/ivars. In summary, there were significantly higher concentrations of fucoxanthin during February 2004 relative to other time periods (Table 2, ANOVA, log transformed, DF=20, F=27.62, p=0.0001). This is consistent with the high concentrations of chlorophyll during February 2004. Additionally, December 2004 had significantly lower concentrations of 19-hexanoyloxyfucoxanthin (*Phaeocystis* indicator accessory pigment) relative to December 2001 and 2003. The presence of more *P. antarctica* during December 2001 and December 2003 is confirmed with microscopic observations in Section II (Table 2, ANOVA, log-transformed, DF=25, F=48.12, $p<0.0001$).

Nutrient Budgets

In spring 2001–2002 the integrated nitrate uptake equaled 0.66 mol m^{-2} which is equivalent to a November–December net community productivity of 1.11 g C m^{-2} d^{-1} (Table 3). Similarly, for the entire growing season (120 d), the total nitrate removal was 0.85 mol m^{-2}, which is equivalent to net productivity of 0.70 g C m^{-2} d^{-1}. Summer productivity was thus 0.31 g C m^{-2} d^{-1} (Table 3). In a similar manner, nitrate uptake in 2003–2004 for spring, summer and for the entire season equaled 0.54, 1.65 and 2.19 mol m^{-2}, respectively. This is equivalent to a daily carbon productivity of 0.94, 2.71, and 1.82 g C m^{-2} d^{-1}, respectively.

Silicic acid removal in spring of 2001–2002 was 0.43 mol m^{-2}, and for summer it was 1.38 mol m^{-2} (total net removal for the entire season was 1.81 mol m^{-2}; Table 3). This is equivalent to diatom productivity for austral spring, summer and the entire 2001–2002 season of 0.19, 0.59, and 0.39 g C m^{-2} d^{-1}, respectively. Thus, diatoms accounted for only 17% of the productivity in spring, but 55% of the total seasonal productivity. Diatom silicic acid removal in 2003–2004 for spring, summer and the entire year equaled 0.74, 4.68 and 5.41 mol m^{-2}, respectively, which, when converted to carbon units was 0.32, 1.99, and 1.16 g C m^{-2} d^{-1}, respectively (Table 3). This represents 35, 73 and 64% of the spring, summer and seasonal production, respectively. During austral spring of December 2004, nitrate and silicic acid uptake was larger than 2001 and 2003 at 0.86 and 0.82 mol m^{-2}, respectively. This was equivalent to 1.53 g C m^{-2} d^{-1} carbon productivity and 0.36 g C m^{-2} d^{-1} diatom carbon productivity. Summer nitrate and silicic uptake rates were much lower in 2004 than February 2002 and 2005 with 0.14 mol m^{-2} nitrate uptake and 0.06 mol m^{-2} silicic acid uptake. With the nutrient uptake being so low, the carbon productivity

was 0.16 g C m^{-2} d^{-1} and diatom productivity 0.03 g C m^{-2} d^{-1}. Although nitrate uptake was low in summer of 2004-2005, nitrate uptake over the entire season was larger than 2001-2002 with 1.00 mol m^{-2} and carbon productivity of 0.74 g C m^{-2} d^{-1}. Diatom productivity was much lower for the entire season (2004-2005) at 0.19 g C m^{-2} d^{-1}.

Uptake Ratios

Si:N uptake ratios were calculated using the integrated nitrate and silicic acid removal estimates (Table 4). In 2001–2002, the ratio was low in spring (0.66), but increased by an order of magnitude during summer (to 7.13), so that the seasonal ratio was 2.13. Spring, summer and seasonal Si:N uptake ratios in 2003–2004 were 1.36, 2.84 and 2.47, respectively. S:N uptake ratios were low throughout the entire growing season in 2004-2005 with a entire season uptake ratio of 0.88. Because the particulate nitrogen and biogenic silica concentrations were measured at the same depths and locations where nutrients were assessed, it is possible to compare the nutrient removal estimates with the particulate matter concentrations at that time, and calculate the amount of material that was lost from the upper 200 m either via transformation to reduced, dissolved forms (for nitrogen) or by export from the surface layer as particles. In spring (the period from early November through our sampling in late December) 2001–2002, "export" equaled 0.16 and 0.27 mol m^{-2} of nitrogen and silicic acid, and in summer (the period from late December through our sampling date in February) it increased to 0.48 and 1.15 mol m^{-2} (Table 4). In contrast, export in 2003–2004 in spring was 0.18 and 0.58 mol m^{-2} for nitrogen and silicon, and in summer 1.75 and 4.12 mol m^{-2} (Table 4). Thus, on a seasonal basis silicic acid and nitrogen export were 4.1 and 4.0 times greater in 2003–2004 than in

2001–2002, respectively. Export during 2003-2004 was also higher than in 2004-2005 being 6.35 and 3.03 times greater for silicon and nitrogen export.

DISCUSSION

Temporal variations in physical parameters

While a full quantitative assessment of the interannual variations within the southern Ross Sea cannot be derived from the data of these three years alone, the observations do provide insights regarding the extent and nature of these variations. Comparison of the hydrographic data shows that mixed layer depths increased from December through February which is similar to the results of Smith and Asper (2001), who found that Z_{mix} was minimal in late December (22.6 m) and increased through January. Smith et al. (2000) found that the minimum Z_{mix} was between mid-December and early January (no sampling occurred between those two dates), and that mixed layer depths increased slightly through February. Mixed layer depths were not significantly different in December 2001, 2003, and 2004 (Table 1). Mixed layer depths during February of 40 m are close to the maximum suggested by Mitchell and Holm-Hansen (1991) as being the deepest mixing that would still support positive community photosynthesis, and so it is quite possible that at some of the stations light limitation of phytoplankton growth was occurring. There was no apparent relationship between large-scale ice distribution and interannual variations in phytoplankton biomass and assemblage composition (Smith et al., 2006). In 2001–2002 ice concentrations were "normal" and similar to the long-term trends with regard to the timing of rapid ablation and the area uncovered (Kwok and Comiso, 2002; Smith et al., 2006). This gave rise to a

high biomass, *P. antarctica*-dominated bloom in spring followed by an assemblage in which diatoms contributed a majority of the chlorophyll. The season 2003–2004 was a heavy ice year, with reduced open-water concentrations in all regions (and especially near 75° S, 170°W, both on and off the continental shelf), but with a large *P. antarctica* bloom in spring and a secondary, very large diatom bloom by February (Table 2). Indeed, the secondary bloom was an order of magnitude greater than the February climatology (Smith et al., 2003a). It has been frequently suggested that *P. antarctica* is favored under low-irradiance conditions (via deeper mixed layers) when compared to diatoms (Moisan and Mitchell, 1999; Arrigo et al., 1999; Smith and Asper, 2001); however, on the coarse scale of our analysis, ice cover (and hence irradiance) did not seem to consistently structure community composition (Smith et al., 2006). Further analysis in Section II suggests that sea surface temperature or increased water mass intrusions may play a role in structuring phytoplankton assemblages. We sampled ice-free waters more completely, and so the generalized description of assemblage composition refers only to those waters with low ice concentrations. It does suggest, however, that irradiance is only one factor controlling biomass and composition of the Ross Sea phytoplankton.

Nitrate concentrations during the three years varied substantially, and with concentrations in the surface layer in February 2004 were significantly lower than February 2001 and 2005 (Figure 3a-f, Table 2). The mean concentration of silicic acid in February 2004 was also significantly less than in 2001–2002. This suggests that silicic acid removal in during spring of the growing season of 2001–2002 was reduced relative to the long-term mean. Variations in relative nutrient uptake can be best explained by variations in phytoplankton species composition effects, but also are influenced by

variations in Si/N uptake ratios. Changes of phytoplankton community composition potentially could alter both food webs. It has been suggested that *P. antarctica* is largely ungrazed and is remineralized within the water column (DiTullio and Smith, 1996; Caron et al., 2000).

Data from other studies collected in approximately the same area can be directly compared to data obtained during this study. Smith and Asper (2001) found the mean nitrate and silicic acid concentration during late December 1995 to be 20.7 and 63.4 μM (both less than those in 2001), with minima being 12.5 and 51.6 μM, respectively. Mean NO_3 concentrations in 1997 were 12.5 μM which were significantly less than in 2001/2002, but were similar to those measured in 2004. Clearly substantial interannual variations in nitrate concentrations occur as a result of variations in the uptake of nitrate by phytoplankton, and hence the question then arises as to the causal mechanism of these differences. Further statistical analysis in Section II will help us ascertain what some of these mechanisms may be.

Nutrient budgets

Removal of nitrate and silicic acid varied substantially across years and seasons. Nitrogen uptake in spring was similar between the three years (0.66, 0.54, and 0.86 mol m^{-2}). Summer nitrate was far greater in February 2004 than February 2001 (1.65 vs. 0.19 mol m^{-2}, more than an 8-fold increase). Silicic acid uptake was similar between the two years (0.43, 0.74, 0.82 mol m^{-2}) in spring, but again summer February 2004 uptake was higher 2001–2002 and 2004-2005 (Table 3). The difference is likely due to a difference in biological removal which, in turn must have been related to both overall controls of productivity (light or iron limitation) as well as the types of phytoplankton taxa present.

Uptake ratios of silicon:nitrogen can be affected by two independent factors: influence by nonsiliceous phytoplankton on the uptake of nitrogen, and alteration of the Si:N uptake ratio by trace metal limitation. One of the major phytoplankton species in the southern Ross Sea is *Phaeocystis antarctica*, and its presence would result in a removal of nitrate in the absence of the removal of silicic acid. As *P. antarctica* often is found in substantial quantities in spring, $Si(OH)_4$:NO_3 uptake ratios would be expected to be relatively low. Hutchins and Bruland (1998) and Takeda (1998) found that iron limitation elevates the $Si(OH)_4$:NO_3 uptake in diatoms by suppressing nitrate uptake while silicon uptake proceeds at relatively normal rates. Furthermore, iron limitation has been experimentally observed in the southern Ross Sea by a variety of means (Sedwick and DiTullio, 1997; Sedwick et al., 2000; Olson et al., 2000). Our $Si(OH)_4$:NO_3 uptake ratios were low (less than or equal to 1) in spring of 2001 and 2004, consistent with both the large contribution of *P. antarctica* and/or the absence of iron limitation. In 2004-2005, there was a large presence of diatoms when we arrived to the study area. However, uptake ratios increased in summer of 2002 and 2004 (Table 4), and in 2001–2002 the ratio exceeded 7 (in nutrient replete cultures the ratio is near 1; Takeda, 1998). Such elevated ratios are commonly found only in highly iron-stressed systems, and we suggest that in the summer of 2001–2002 low levels of iron limited the diatom assemblage. Growth did not cease, as evidenced by the continued (albeit slow) reduction in nitrate (by nearly 3 μM during summer in the surface layer), but growth was substantially reduced. *P. antarctica* could not have replaced diatoms, as its iron requirements appear greater than those of the diatoms of the region (Coale et al., 2003); indeed, it is possible that iron limitation and stress of *P. antarctica* may be a cause for increased losses due to passive

sinking of colonies, aggregation, and disruption of colonies (Smith and Asper, 2001; Smith et al., 2003b).

The Si:N uptake ratio in summer 2003–2004 increased to 2.84 which, also indicates the potential for iron-stress, but our data suggest that the growth of diatoms was not greatly influenced and that they continued to increase in biomass. Chlorophyll levels also were higher in February when compared to December (Table 2, Figure 5a-f). We believe diatoms largely drove this increase. Such a substantial and unexpected increase (based on the climatological chlorophyll distribution which decreased during February; Table 2, Smith et al., 2003) suggests that iron limitation was not great enough to greatly reduce growth, and suggesting that iron inputs to the region may have been greater in 2003–2004 than in 2001–2002. Although the hydrographic data from 150 m do not contain anomalous data, a more detailed analysis of the data from 50 to 200 m shows pockets of what appears to be modified circumpolar deep water (MCDW) (Peloquin, 2005). MCDW may be a source of heat and iron and is characterized by being warmer (-0.5-1°C), more saline (34.4–34.5) and relatively micronutrient-rich compared to surrounding waters (Hiscock, 2004). The intrusion of MCDW onto the Ross Sea continental shelf has been reported previously (Jacobs et al., 1995), and has been suggested to influence the timing and magnitude of the seasonal phytoplankton bloom by providing iron to surface waters (Hiscock, 2004; Arrigo and van Dijken, 2004). An inconsistency in using this hypothesis is that stratification was still strong in February, which would restrict the introduction of the Fe-replete waters to the euphotic zone. However, in a fine resolution study, Hales and Takahashi (2004) detected small expressions of this water mass in Ross Sea surface waters, suggesting that mesoscale

inputs of iron occur and may fuel blooms. Therefore, we believe that the initial *P. antarctica* accumulation and growth was fueled by iron supplied via deep winter mixing, and that the secondary bloom dominated by diatoms was initiated by the introduction of MCDW into the euphotic zone. During December 2004, water mass intrusions were also present (Peloquin, unpublished, Section II). However, in February 2005 surface nutrient concentrations increased; therefore, there must be an intrusion of nutrient rich water into the area or mixing. It is not known at this time whether the phytoplankton assemblage present in February 2005 was iron limited, even though Si:N ratios for the summer were low. However, since diatoms were present in large numbers during December of that season, this may have affected micronutrient concentrations in February.

Export

Export of biogenic material from the surface layer (200 m), calculated by mass balance, also showed substantial differences among years (Table 4). Nitrogen disappearance was 4-fold greater in 2003–2004 than in 2001–2002 for the entire season, although in spring the export was only 2.1-fold greater. Nitrogen export was also lower in 2004-2005, with nitrate and silicic acid export being 3.03 and 6.35-fold less than 2003-2004. Comparison with the observations of Sweeney et al. (2000b) indicates that 1996–1997 had a nitrogen removal (1.33 mol m^{-2}) intermediate between 2001–2002 and 2003–2004, whereas the silicon deficit (0.47 mol m^{-2}) was more than an order of magnitude less than we observed in 2003–2004. This suggests that diatoms were far more important in the biogeochemical budgets during the period of our observations, even though the exact reasons for these differences cannot be definitively ascertained. Our seasonal estimates of export can be roughly compared to those obtained by sediment traps. The comparison is

inexact, because our estimates are based on changes in NO_3^- concentrations, and nitrate can be reduced into dissolved pools (NO_2^-, NH_4^+, DON) in addition to reduced particulate pools (PN). By not accounting for DON and NH_4 increases, particulate export is overestimated. DON net production in the upper 150 m was estimated by Carlson et al. (2000) to be ca. 9% of PN production. Ammonium production by mid- January was ca. 2% of PN production (Smith and Asper, 2000), but Gordon et al. (2000) reported late summer NH_4^+ values that were approximately double those of Smith and Asper (2000). To complicate the budget further, NH_4^+ is rapidly used by phytoplankton in the surface layer, so that ammonium budgets will underestimate the true fluxes through this pool. Based on these uncertainties, the production of dissolved, reduced N (the sum of ammonium and DON) likely is ca. 20% of particulate production. This is not insignificant, but also is likely to be the maximum influence on the total N budget. Silicic acid has no reduced phases and is not influenced by the same biological processes.

The particulate flux data of Collier et al. (2000), for the same season as the nutrient deficit results of Sweeney et al. (2000b), show that during austral spring 1996/7, N flux at 206 m was 0.67 mol m^{-2}, and that Si fluxes were ca. 0.34 mol m^{-2} (assuming a C/Si ratio of 0.1 in early season and calculating the flux from November 1). N and Si fluxes through February 8 equaled approximately 3.34 and 3.81 mol m^{-2}, respectively. These are higher than estimates of export of nitrate and silicic acid in our study, but it should be noted that in both years we found a substantial amount of biogenic matter still within the water column. Presumably this material ultimately would be either remineralized or contribute to particulate flux to depth. Nearly all sediment traps in the Ross Sea have detected a significant temporal decoupling between production and

vertical flux (Nelson et al., 1996; Smith and Dunbar, 1998; Dunbar et al., 1998; Collier et al., 2000), and our particulate matter distributions and concentrations would suggest that a substantial amount of export (but not regeneration) of nitrogen and silicon would occur after the last period of our sampling. It remains unclear what the fate of the material exported below 200 m might be. It is possible that the diatomaceous material at that depth will sink rapidly and reach the sediments relatively intact, whereas the organic material in *P. antarctica* might be largely remineralized within the water column (Nelson et al., 1996). Sinking rates of the two forms (siliceous vs. non-siliceous) are likely different (both at the surface as well as at depth), as is the time of appearance in the surface layer. The composition of the surface assemblage influences the material collected in traps at depth (Dunbar et al., 1998), but our results do not extend long enough to resolve the contributions adequately of the quality of surface biogenic material vs. the absolute export.

Spatial variations

An assessment of temporal variations can be obscured by spatial variations. All studies of biomass in the Ross Sea have observed variations not only in phytoplankton biomass, but in assemblage composition, and these variations occur on a variety of scales (Hales and Takahashi, 2004). Many of the spatial variations may indeed be coupled to temporal variations; that is, the time needed for biomass changes is dependent on the spatial distribution of a primary physical constraint like ice (Arrigo et al., 1998). Advection of water could introduce a serious error into our calculations of nutrient drawdown. However, we do not believe that this error was substantial during the time periods of 2001-2002 and 2003-2004. Previous studies that measured net velocities

within our study area found rates ca. 0.6 and 1 cm s^{-1} when measured at 220 and 440 m throughout the year, and even less during summer (Pillsbury and Jacobs, 1985; Picco et al., 1999). Such rates represent a monthly advection of 15.6 and 25.9 km and clearly are small relative to the size of the observed bloom (100's of km; Smith and Nelson, 1985; Arrigo et al., 1999). Similar calculations of transport based on modeled results confirm the relatively minor advective motions (Dinniman et al., 2003). Advective changes large enough to alter the nutrient budgets also should be observed as rapid changes in the continuous record of fluorescence (see discussion: Smith et al. 2006); however, none were observed during 2003–2004. While advection might introduce errors in our calculations, we have no evidence to suggest that this is a significant error in our budgets. The increase in surface concentrations in February 2005, however, might be affected more by physical processes as biomass is extremely low during that time making biological uptake of nitrogen very low. In addition, Smith and Dunbar (1998) found that nitrate uptake decreased in February so the lower biomass combined with these physical effects may be what we are observing in our data set.

Conclusions

Variations among years in the biogeochemical variables and cycles of the southern Ross Sea are large. All three of the years we sampled were in some ways anomalous when viewed in the context of the long-term conditions of the region (Smith et al., 2003a). Blooms were dominated by one species (Diatoms or *P. antarctica*) rather than a mixed assemblage and large diatom blooms were observed in February of 2004. It is impossible to say if the large diatoms blooms observed in February 2004 will be a consistent feature in the future, but we know that these diatom accumulations were much

greater than was observed in studies conducted during the 1990s. Because phytoplankton assemblage composition has such a critical role in food web structure and biogeochemical cycles, knowledge of this variable is critical to our understanding of energetic and elemental cycles of the Ross Sea. Further measurements are needed to establish the role of iron and grazing on phytoplankton composition and biomass in the region. Only with additional time series measurements can we begin to understand and predict the interannual variability and the long-term temporal changes that might occur in the Ross Sea as a result of regional, basin-wide and global change.

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Table 1. Hydrographic properties including mixed layer depth (Z_{mix}) in December and February and December values of surface temperature (T), Salinity (S), and Density (σ_t) for IVARS Stations during 2001-2005. Bold values indicate a significant difference between years.

Year	n	December Z_{mix} (m)	February Z_{mix} (m)	$T_{surface}$ °C	$S_{surface}$ psu	$\sigma_{t\ surface}$
2001-2002	5	23.5 ± 8.8	36.6 ± 14.07	-1.0 ±0.2	34.4 ± 0.1	27.7 ± 0.07
2003-2004	6	19.1 ± 8.6	20.4 ± 10.8	-0.9 ± 0.3	**34.1 ± 0.1**	**27.5 ± 0.06**
2004-2005	20	21.7 ± 6.5	38.7 ± 15.6	**0.1 ± 0.6**	34.4 ± 0.1	27.7±0.04

Table 2. Average surface nutrient (Nitrate, Silicic acid, and phosphate) and pigment (Fucoxanthin and 19 –hexanoyloxyfucoxanthin) concentrations for IVARS transect stations. Bold values indicate a significant difference between years.

Month/Year	n	NO_3 (μM)	$Si(OH)_4$ (μM)	PO_4 (μM)	Chlorophyll ($\mu g\ L^{-1}$)	Fucoxanthin ($\mu g\ L^{-1}$)	19 -hex ($\mu g\ L^{-1}$)
December 2001	5	22.0 ±1.3	**77.2 ± 1.7**	**1.8 ± 0.1**	5.8 ± 1.9	1.8 ± 0.6	9.3 ± 2.6
February 2002	5	20.5 ± 1.6	65.4 ± 2.6	1.6 ± 0.1	**4.9 ± 2.2**	**1.2 ± 0.4**	0.8 ± 0.9
December 2003	6	20.0 ± 1.7	66.6 ± 4.8	**1.6 ± 0.1**	5.4 ± 3.5	1.5 ± 0.6	4.0 ± 3.1
February 2004	7	**16.8 ± 2.1**	**45.8 ± 5.0**	1.6 ± 1.9	**11.1 ± 4.3**	**5.6 ± 2.4**	0.8 ± 0.3
December 2004	17	**16.5 ± 3.0**	63.5 ± 8.7	**1.1 ± 0.1**	7.9 ± 3.9	3.8 ± 3.4	**0.3 ± 0.3**
February 2005	11	19.7 ± 1.9	66.2 ± 10.4	1.3 ± 0.1	**1.0 ± 0.7**	**0.4 ± 0.5**	**0.2 ± 0.2**

Table 3. Net nitrate and silicic acid removal (and standard deviations) of phytoplankton as estimated by Eqs. (1) and (2), and the computed carbon equivalent production of the entire assemblage and of diatoms.

Date and Period	Nitrate uptake ($mol\ m^{-2}$)	Carbon productivity ($g\ C\ m^{-2}\ d^{-1}$)	Silicic acid uptake ($mol\ m^{-2}$)	Diatom carbon productivity ($g\ C\ m^{-2}\ d^{-1}$)
Spring, 2001[a]	0.66±0.74	1.11	0.43±0.75	0.19 (17%)
Summer, 2001-2002[d]	0.19[c]	0.31	1.38c	0.59 (188%)
Entire Season, 2001-2002[b]	0.85±0.27	0.7	1.81±0.66	0.39 (55%)
Spring, 2003[a]	0.54±0.22	0.94	0.74±0.30	0.32 (35%)
Summer, 2003-2004[d]	1.65[c]	2.71	4.68	1.99 (73%)
Entire Season, 2003-2004[b]	2.19±0.30	1.82	5.41±1.13	1.16 (64%)
Spring, 2004[a]	0.86±0.29	1.53	0.82±0.42	0.36 (24%)
Summer, 2004-2005[d]	0.14[c]	0.16	0.06c	0.03 (19%)
Entire Season, 2004-2005[d]	1.00±0.28	0.74	0.88±0.23	0.19 (25%)

[a] Growing season assumed to be from November 1 to December 26 (55 days); f-ratio assumed to be 0.8 for both nitrogen and silicon.
[b] Growing season assumed to be from November 1 to February 28 (120 days); f-ratio assumed to be 0.7 for both nitrogen and silicon.
[c] Calculated by difference.
[d] Growing season assumed to be from December 27 to February 28 (65 days); f-ratio assumed to be 0.75 for both nitrogen and silicon.

Table 4. Ratios of integrated nitrate to integrated silicic acid uptake, nitrogen export, and silicon export during spring, summer, and the entire growing season.

Year/Season	$Si(OH)_4$:NO_3 uptake mol/mol	Nitrogen export (mol m^{-2})	Silicon export (mol m^{-2})
2001-2002, Spring	0.66	0.16	0.27
2001-2002, Summer	7.13	0.32	0.88
2001-2002, Entire Season	2.13	0.48	1.15
2003-2004, Spring	1.36	0.18	0.58
2003-2004, Summer	2.84	1.75	4.12
2003-2004, Entire Season	2.47	1.94	4.7
2004-2005, Spring	0.94	0.45	0.43
2004-2005, Summer	0.51	0.19	0.31
2004-2005, Entire Season	0.88	0.64	0.74

Figure 1. Station locations for Interannual Variations in the Antarctic- Ross Sea (IVARS) for 2001-2005.

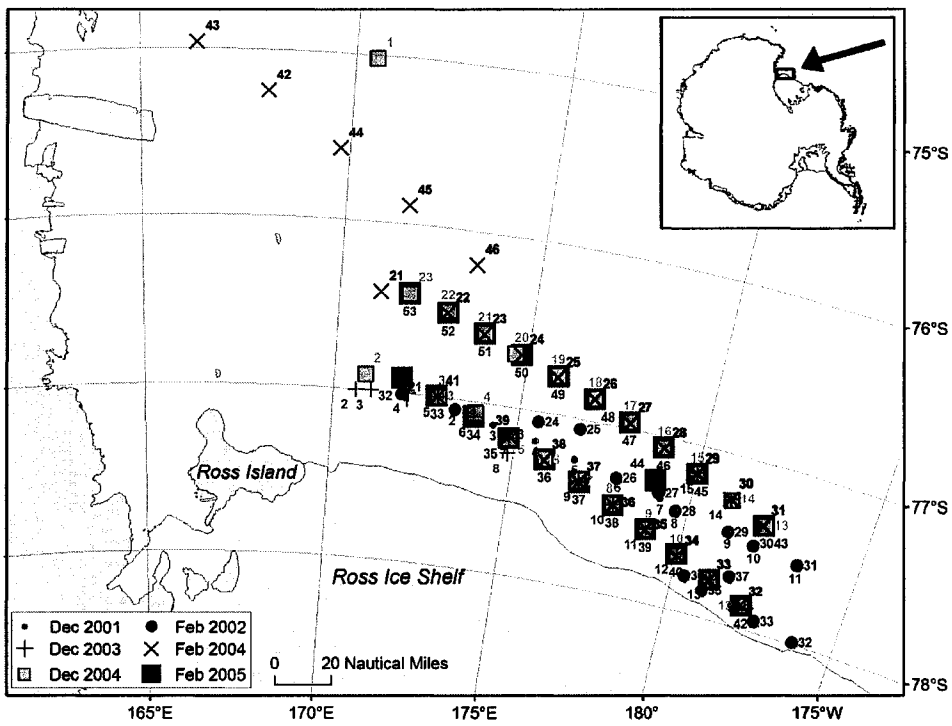


Figure 2. Ice coverage and distributions in mid December, mid-February in 2003,2003,2004,2005. Data from December 2002 and February 2003 not included in this dissertation; from Smith et al. 2006.

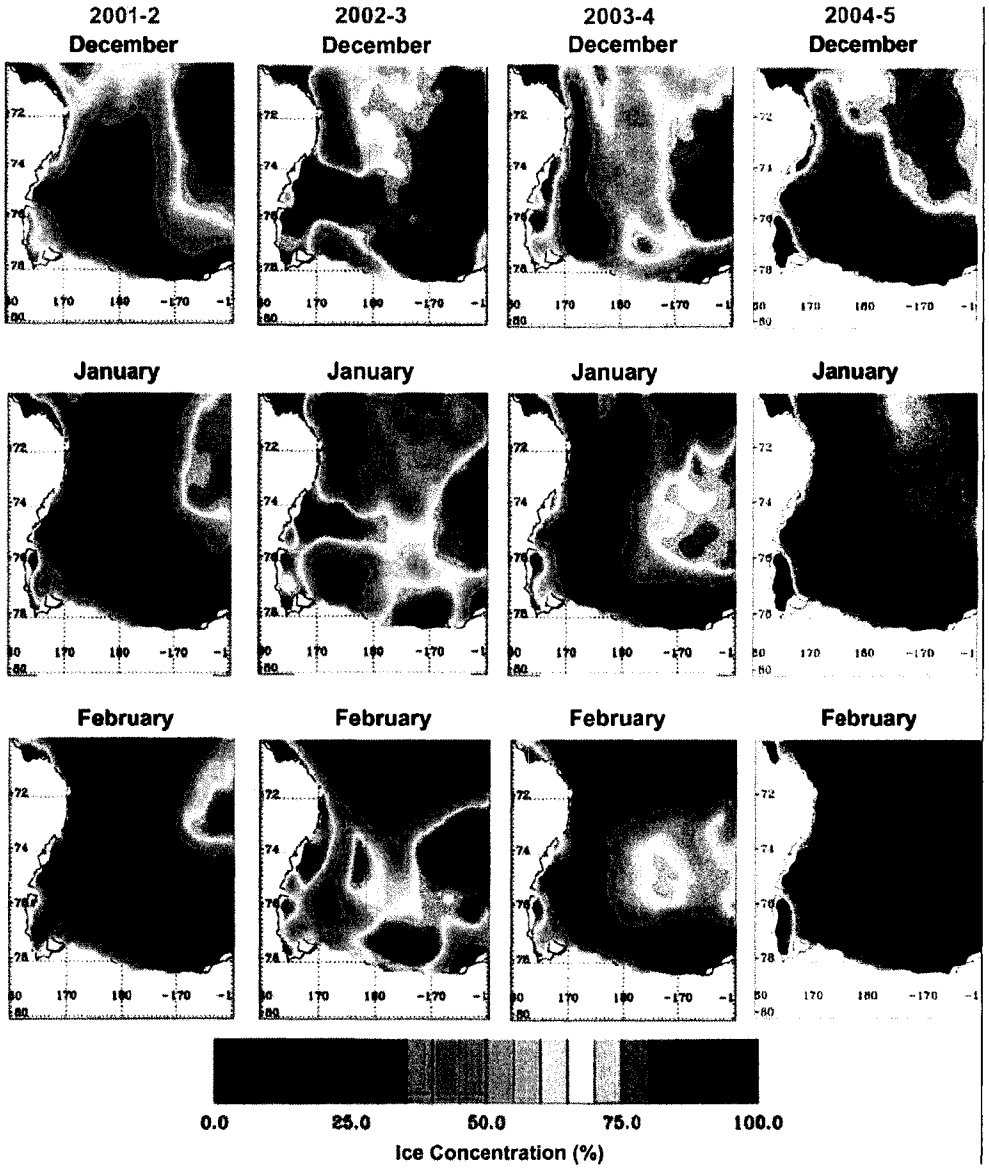


Figure 3. Nitrate distributions (units= μM) along southern transect in (A) December 2001, (B) February 2002, (C) December 2003, (D) February 2004, (E) December 2004, and (F) February 2005.

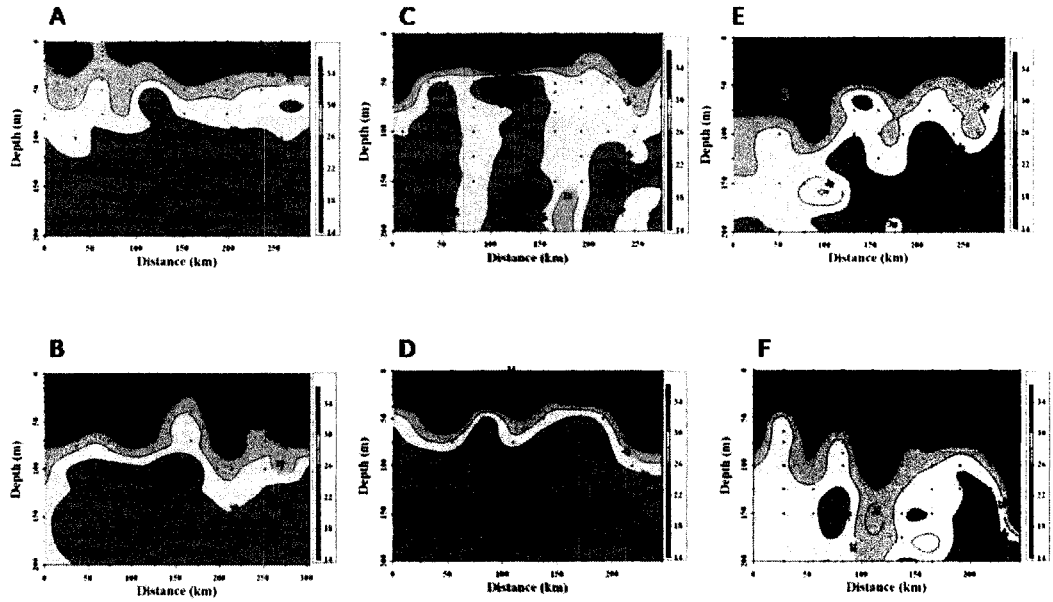


Figure 4. Silicate distributions (units=μM) along southern transect in (A) December 2001, (B) February 2002, (C) December 2003, (D) February 2004, (E) December 2004, and (F) February 2005.

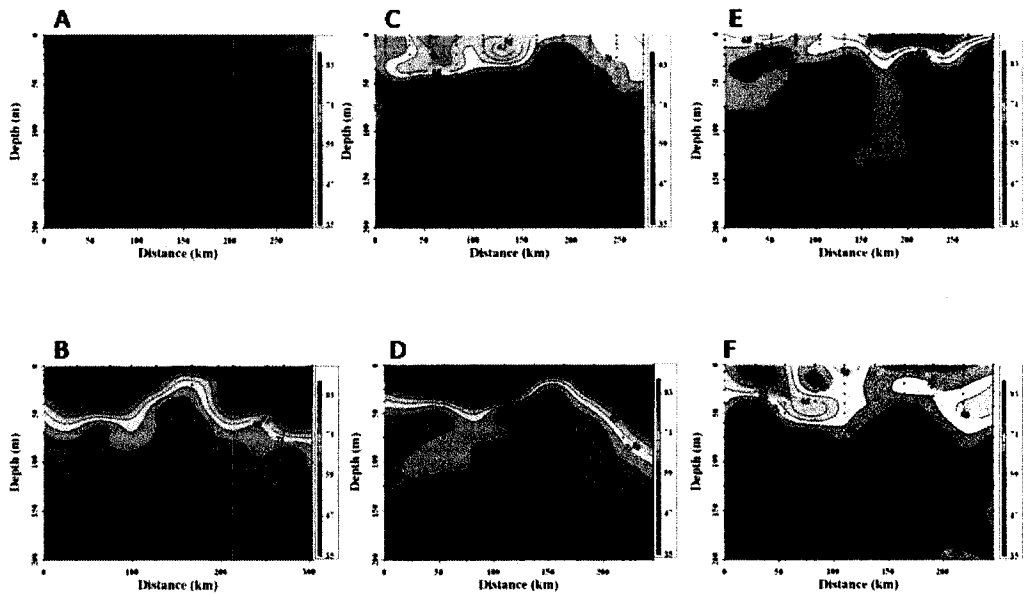
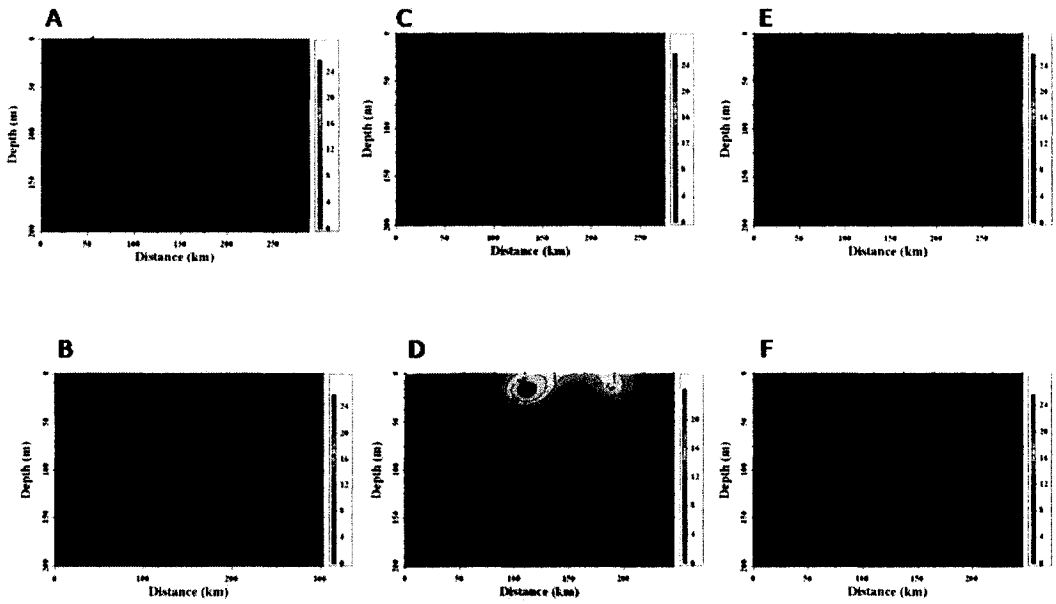


Figure 5. Chlorophyll a distributions (units= $\mu g\ L^{-1}$) along southern transect in (A) December 2001, (B) February 2002, (C) December 2003, (D) February 2004, (E) December 2004, and (F) February 2005.



Section II. The role of sea surface temperature in regulating nitrate removal in *Phaeocystis antarctica* dominated assemblages in the Ross Sea

ABSTRACT

Energy flow within the Ross Sea food web is dominated by two major functional groups of phytoplankton: diatoms and prymnesiophytes, the latter dominated by *Phaeocystis antarctica*. The Ross Sea polynya experiences one of the largest phytoplankton blooms in the Southern Ocean due to the physical properties of annual sea ice retreat and water stratification. As part of a multi-year project, Interannual Variations in the Antarctic-Ross Sea (IVARS), we investigated physical, chemical, and biological factors that could drive phytoplankton taxonomic composition and nutrient uptake in late austral spring. Principal components analysis (PCA) was performed on three years of data including dissolved nutrients (N,P,Si), size fractionated chlorophyll, depth of sample, biogenic silica, particulate organic carbon, particulate nitrogen, temperature, and phytoplankton pigments. While water column depth and seasonal factors are the primary controls on phytoplankton biomass in the Ross Sea, one of the PC explained 13% of the variation in the data set, and this PC correlates with temperature. Further regression analysis of only surface samples shows a significant relationship between increases in sea surface temperature and phytoplankton nutrient removal. However, *P. antarctica* abundance decreases with increases in sea surface temperature, which may be due to an increase in diatom growth. These interannual differences in temperature could be due to water mass intrusions suggesting that physical processes within the Ross Sea may be driving phytoplankton assemblage composition and nutrient removal.

INTRODUCTION

Ross Sea, Antarctica

The Ross Sea polynya, located on the continental shelf of Antarctica experiences relatively predictable phytoplankton blooms due to the physical properties of annual sea ice retreat and water stratification. The formation of the Ross Sea polynya in austral spring is generated by two factors, including southerly winds driving ice northward and exposing open water and the advection of warm (≈-0.5°C) Antarctic Circumpolar Current water onto the shelf, providing a heat source to the surface layer (Markus, 1999). As a coastal and continental shelf zone (CCSZ), hydrologic structure and irradiance are major controls of nutrients and biological activity (Catalano et al., 1997). Seasonal variability and thermohaline characteristics are influenced by total winter ice coverage, ice melting, and fast ice formation. Two types of water masses are present in the Ross Sea, including High Salinity Shelf Water which, is characterized by maximum salinity due to continuous freezing and ice formation and Ice Shelf Waters (ISW) which, have minimum temperatures. Circumpolar Deep Water (CDW) is the only external water mass that affects the Ross Sea region and is separated through the Antarctic Slope Front located near the Continental Shelf Break. Modified Circumpolar Deep Water (MCDW) results from the CDW water mass mixing with surface and shelf waters in the Ross Sea (Budillion et al., 2000). Other local water masses observed include the Antarctic Surface water mass (AASW) which, has temperatures of ~1°C (Jacobs et al., 1985; Jacobs and Giuvili, 1998; Gordon et al., 2000).

Phytoplankton ecology in the Ross Sea

Primary production, vertical flux, and carbon and sulfur transformations are dominated primarily by *Phaeocystis antarctica,* a colonial prymnesiophyte (Smith et al., 2003). Phytoplankton populations in the Ross Sea are dominated by two main algal groups: diatoms and prymnesiophytes, with the latter being dominated by *P. antarctica.* This region is also the most productive region in the Southern Ocean according to biomass and production estimates (Sedwick and DiTullio, 1997; Dunbar et al., 1998; Asper and Smith, 1999; Tremblay and Smith, 2007). Mixed layer depths and micronutrient concentrations may explain the dominance of one taxonomic group, but attempts to find controlling factors have been inconclusive (Smith and Asper, 2001; van Hilst and Smith, 2002). These high accumulations of biomass have been attributed to stabilization of the water column and low grazing pressure during the growing season.

Phytoplankton growth begins in late October (Smith and Gordon, 1997) and is largely limited by irradiance during austral spring (Smith et al., 2000) and by micronutrients (iron availability) in the summer (Fitzwater et al., 2000; Sedwick et al., 2000; Olson et al., 2000). Macronutrients remain high throughout the region for the entire year (Smith et al., 2003). Understanding environmental and oceanographic controls on the composition of phytoplankton assemblages is critical because the taxonomic structure is a major determinant of energy flow within food webs through inorganic carbon fixation and autotrophic energy production (Palmisano and Sullivan, 1985). Diatoms and *Phaeocystis* also have different impacts on biogeochemistry and nitrogen transformations (Smith and Asper, 2001). The size structure of phytoplankton assemblages also drives

marine pelagic food web dynamics (Legendre and Le Fèvre, 1991) and potential export (Tremblay and Legendre, 1994).

Variations in biogeochemical properties, seasonal cycles of phytoplankton biomass, and productivity still remain unresolved in the Ross Sea. A recent study by Smith et al. (2006) noted large interannual variations in biogeochemical variables and cycles and determined these were due to wide ranging physical, biological, and chemical controlling mechanisms. Seasonal differences within growing seasons were thought to be controlled by irradiance in the spring and micronutrients in the summer (Smith et al., 2000).

Temperature and phytoplankton growth

In polar environments biogeochemical cycles could be directly affected by changing temperatures on a global scale through changing the time and duration of wintertime sea ice which, in turn affects the food web, its productivity, and the distribution of upper-trophic level consumers. (Hunt et al., 2002). Phytoplankton growth during large phytoplankton blooms is controlled by nitrate sources beneath the euphotic zone. A recent study suggested that phytoplankton biomass and growth will likely decrease as the oceans warm and stratify throughout the world since nutrient-rich water is found deeper in the water column (Behrenfeld et al., 2006). However, in polar regions, warmer surface seawater temperatures may cause increases in biomass due to the stabilization of the mixed layer and increased light availability (Behrenfeld et al., 2006). Temperature is important in nitrogen assimilation enzyme activity and metabolism (Eppley, 1972; Gao et al., 2000), and a negative relationship has been observed between temperature and nitrate uptake (Lomas and Glibert, 1999). Lomas and Glibert (1999)

conclude that if temperature along with other non-nutritional mechanisms of the cell are regulating nitrate uptake then this must be included in new production models. This relationship between nitrate and temperature has not been fully described in the Ross Sea since little is known about the temperature sensitivity of phytoplankton nitrogen uptake in the Southern Ocean or the Ross Sea, although a higher affinity for ammonium rather nitrate has been suggested (Dortch, 1990; Reay et al., 1999; Cochlan et al., 2002). There is a greater energetic cost associated with the transport, reduction, and assimilation of NO_3^- (McCarthy and Carpenter, 1983). The high *f*-ratios (new production) of the Ross Sea, however, show that ice edge phytoplankton blooms are utilizing nitrate to a greater degree than ammonium (Smith and Nelson, 1990; Hu and Smith, 1998; Cochlan et al., 2002). High half saturation constants (K_s) for silicic acid and nitrate uptake have been reported for Southern Ocean phytoplankton growing at low temperatures, and studies show a strong temperature dependence of nitrate reductase activity (e.g. Tischner and Lorenzen, 1981; Jacques, 1983; Sommer, 1986; Cochlan et al., 2002).

Phytoplankton in the Southern Ocean are increasingly being viewed as psychrotolerant rather than psychrophilic as their optimum growing temperatures are higher than what they experience *in situ* (Tilzer et al., 1986; Smith and Harrison, 1991; Arrigo, 2007). The reason for psychrotolerant species dominance may also be due to other unique adaptations of photosynthetic microorganisms, including *P. antarctica*, which, include the ability to acclimate to extremes in light (from total darkness to photoinhibition) and outcompete psychrophilic phytoplankton, but this research is in initial stages (Shields, unpublished). Assessing these relationships and adaptations are

critical to looking at how environmental controls will affect the biology and chemistry of the Ross Sea and the capacity of phytoplankton to respond to environmental change.

Objectives

As a part of a multi-year project, Interannual Variations in the Antarctic-Ross Sea (IVARS), principal components analysis (PCA) was performed on three years of the late austral spring and summer data set including dissolved nutrients (N,P,Si), size fractionated chlorophyll (>20, >0.7, and <20 μm), water depth (0, 20, and 50 m), biogenic silica, particulate organic carbon, particulate nitrogen, temperature, and phytoplankton pigments. I hypothesized that temperature would structure biomass distributions, phytoplankton composition, and nutrient uptake in the early growing season of December 2001, 2003 and 2004. I also expected to see seasonal differences in biogeochemical parameters due to stratification and possible micronutrient limitation. Furthermore, I expected to observe a spatially extensive and predictable phytoplankton bloom (Arrigo and McClain, 1994) and that temperature would play a role in bloom composition (diatoms vs *P. antarctica*). Previous studies have investigated variations in phytoplankton biomass, nutrients, salinity, and temperature in the past, but the samplings were not distributed evenly over time and space, and it was hard to draw conclusions about the relationship between these variables. This study is the first to show a clear relationship between temperature and austral spring bloom dynamics and will help modelers and scientists predict how future changes in the ecosystem due to increases in surface water temperature will affect the food web of the Ross Sea. It should be noted, however, that temperature may be a proxy for other factors, such as water mass intrusions which may introduce warmer temperatures along with micronutrients such as iron.

METHODS (some excerpts from Section I)

Sample collection

Water samples were collected from the southern Ross Sea during three field seasons (2001–2002, 2003-2004, and 2005-2006) in late spring (mid- to late December) and late summer (mid-February) using the USCGC *Polar Star* and RVIB *N.B. Palmer*. Station locations were part of a transect that was roughly parallel to the Ross Ice Shelf, but were largely chosen to sample ice-free waters (Figure 6; Smith et al., 2006). Samples were collected using 10-L Niskin bottles mounted on a rosette frame which also housed a SeaBird 911+ CTD and Chelsea fluorometer to collect continuous profiles of temperature, salinity, density, and fluorescence during water sampling from discrete depths. A total of 12 depths were sampled to 200 m. (For detailed methodology of sampling depths and processing, see Smith et al, 2006). Temperature, salinity and derived density data were binned into 1-m intervals, and mixed layer depths derived from the vertical distribution of density (Z_{mix} was defined as the depth where σ_T changed by 0.1 units from a stable, surface value; Smith et al., 2000). Complete hydrographic data are available at http://www.vims.edu/bio/ivars/.

Samples were collected for nutrients (nitrate+nitrite, silicic acid, phosphate), chlorophyll (size fractionated: >5 μm, >20 μm, and total), pigments, particulate nitrogen (PN), particulate organic carbon (POC) and biogenic silica (BSi). Samples (60 mL) for nutrients with elevated chlorophyll levels (those with approximately 1.0 μg L^{-1} chlorophyll a or more) were filtered through Gelman Acrodiscs (5.0 μm) and frozen at -80 °C for later analysis using standard, automated techniques. Chlorophyll samples were filtered through either polycarbonate (20 or 5 μm; Poretics) or Whatman GF/F filters,

placed in 7 mL 90% acetone, and sonicated for 15 min. After extraction for at least another 15 min on ice in darkness, the filter was removed and the sample read on a Turner Designs Model AU fluorometer before and after acidification. The fluorometer was calibrated before and after the cruise using commercially purified chlorophyll (Sigma) and checked using high-performance liquid chromatography. PN and POC were collected by filtering known volumes of water through precombusted GF/F filters, rinsed with ca. 5 mL 0.01N HCl in seawater, and drying the filters in combusted glass vials at 60°C. Blanks were filters placed under the deep-water sample's filter; these filters were processed identically to the other samples. All filters were analyzed using a Carlo–Erba Model 254 elemental analyzer (Smith et al., 1996). Samples for biogenic silica were filtered through 0.6 μm Poretics polycarbonate filters, dried in plastic Petri dishes at 60°C, and returned to the laboratory. The samples were then digested in NaOH at 100 °C for 40 min, neutralized and analyzed colorometrically for reactive silica on a dual-beam spectrophotometer (Brzezinski and Nelson, 1989). Contributions of lithogenic Si to total BSi are small in the Ross Sea (1%; Nelson, unpublished) and were ignored. HPLC sample analysis and collection is described in Section I and Smith et al. (2006).

Data analyses

Analysis of Variance (ANOVA) was used to compare mean abundances in samples collected from different periods. When data did not meet the assumptions of parametric statistics, the data were log transformed to yield homogeneous of variance and normality. Where a significant difference was detected for an ANOVA ($p<0.05$), Tukey's multiple comparison test was used to test for specific differences. Principal components analysis was performed on the whole data set to assess the factors driving variations in

phytoplankton abundance and nutrient uptake in the Ross Sea. Multivariate analysis allows the pattern of relationships between several variables to be viewed simultaneously. PCA also reduces the complexity of the data set and by identifying a few principal components that explain most of the variability of the data set (Zitco, 1994; Meglen, 1992). A number of ecological and biogeochemical studies have successfully used this method to examine the relative importance of bottom-up and top-down factors in phytoplankton assemblage control and in order to understand how different physical parameters affect submerged aquatic vegetation and algae (Metaxas and Scheibling, 1996; Bayley and Prather, 2001). PCA and regression statistics were performed in Minitab version 14.0. Minitab software automatically uses a normalized correlation matrix through the standardization of variables by subtracting the mean and dividing by the standard deviation (Zimmerman and Canuel, 2001). Only stations in which all data were available for PCA analysis were included in this analysis.

RESULTS

Initiation of the bloom varied interannually due to a wide range of environmental conditions over the three year study period. Hydrographic data means showed that the mean mixed layer depths were similar during December 2001, 2003, and 2004, but there were between-year differences in surface temperature, salinity, and σ_T (Table 5). During December 2004 surface temperatures were significantly higher (0.1 ± 0.6 °C) than in 2001 and 2003 (Table 5, ANOVA, DF=28, F=15.10, $p<0.0001$). In December 2003 surface salinity and σ_T values were significantly lower than in December 2001 and 2003 with values of 34.1 ± 0.08 and 27.5 ± 0.06, respectively (Table 5, salinity, ANOVA, DF=28, F=41.60, $p<0.0001$; and σ_T ANOVA, DF=25, F=44.86, $p<0.0001$). Further

analyses are being performed to look at the roles that MCDW and other physical factors, including irradiance and ice melt, play on these varying hydrographic conditions. It is clear, however, that there was significant between-year variation in December bloom conditions during our study period (Table 5).

Interannual variability was also observed in inorganic nutrient concentrations (Table 6). Silicic acid concentrations (77.2 ±1.7 μM) and phosphate (1.8 ± 0.06 μM) were significantly higher in December 2001 (Table 6, silicic acid, ANOVA, DF=25, F=5.96, p=0.0008 and phosphate, ANOVA, DF=25, F=52.0, p<0.0001). Nitrate concentrations (16.5 ± 3.0 μM) and phosphate concentrations (1.1 ± 0.1 μM) were significantly lower during December 2004 (Table 6, Nitrate, ANOVA, DF=25, F=10.93, p<0.0001). These values represent the magnitude of phytoplankton nutrient utilization in the surface waters of the Ross Sea since winter values of nitrate, silicic acid, and phosphate are known. For further discussion of the nutrient removal and budgets, see Smith et al. (2006) and Section I.

While there were no significant interannual differences in bulk chlorophyll or fucoxanthin concentrations, 19 -hexanoyloxyfucoxanthin (19 -hex) was significantly lower in December 2004 with values of 0.3 ± 0.4 μg L^{-1} (Table 6, ANOVA, DF=25,F=48.12, p<0.004). These data suggest that *P. antarctica* concentrations were lowest during 2004. This was confirmed by microscopic observations of cruise samples. During 2004 diatoms comprised >95% of the phytoplankton community abundance (Figure 7; Peloquin et al., in prep.). *P. antarctica* dominated the assemblages during 2001 and 2003 austral spring, but differed in its morphological form. Solitary forms of *P.*

antarctica were most abundant during 2001, while the colonial form was more abundant during December 2003 (Peloquin et al., in prep.; Shields, unpublished).

Principal components analysis

Principal components analysis (PCA) was performed to reduce the complexity of the data set and identify factors controlling variation within the extensive data set. PCA was applied to 19 variables and 151 observations (samples). Together, the first three principal components (PCs) explain 68.1% (37.9%, 16.6%, and 13.6%) of the total variance of the data. The PCs seem to represent interpretive factors that include temporal (seasonal and interannual) variations, biomass and organic matter concentration, water column depth, phytoplankton species composition, and temperature. While we were primarily interested in bloom development in December, data from IVARS stations in December and February were included in order to discern any temporal trends within the data set.

Variable loadings

Loadings for PC1 vs. PC2 and PC1 vs. PC3 were plotted in order to assess relationships between the variables and each PC (Table 7, Figure 8a, 9a). Variables with positive loadings on the PC axis indicate a direct relationship with each PC. The magnitude of the loadings is indicative of the influence of the variable on each PC (Zimmerman and Canuel, 2001). In the PC1 vs. PC2 plots, phytoplankton pigments including chlorophyll, fucoxanthin, PN, and POC projected close to one another in the positive PC1 coordinate space while inorganic nutrients, salinity, and density projected close to one another in the negative loaded PC1 coordinate space (Table 7, Figure 8a). The most negatively loaded (<-0.2) variable on PC1 was nitrate+nitrite while the most

positively loaded was >20 μm chlorophyll. The strong positive loadings of pigments on the PC1 axis and negative loadings of inorganic nutrients suggest that PC1 separates out the data on a depth and range of biomass and organic matter concentrations with the deepest samples having negative loadings and surface samples having positive loadings. Loadings on PC1 might also be affected by other parameters not measured including irradiance which, is an important control on phytoplankton production.

Temperature and year have the most positive loadings on PC2 (>0.15). This suggests that PC2 describes temporal variability among the samples (Table 7; Figure 8a). 19-hex, salinity, density and season had the most negative loadings on PC2 with values < -0.3. PC2 can be interpreted as separating samples based on seasonality and 19-hex (*P. antarctica*) concentrations upon examination of the scores.

Temperature, salinity, and year (loadings of 0.47, 0.41, and 0.38, respectively) had the most positive loadings on PC3 (Table 7; Figure 9a). This may suggest that PC3 resolves between samples collected during high vs. low water temperatures. December 2004 had significantly higher temperatures than 2001 and 2003, and PC3 generally resolved between 2004 samples and samples collected in 2001-2003 (Figure 9b).

Observation Scores

PC scores indicate relationships between the PC and individual samples. PC1 separates the data by station depth in the water column and the concentrations of chlorophyll and organic matter (Appendix A-C; Figure 8b). Samples located deeper within the water column had higher concentrations of inorganic nutrients and lower concentrations of particulate nitrogen, chlorophyll, and other fixed organic matter (Figure 8b). Most of the samples from stations sampled in December had negative PC2 scores,

while February samples had positive PC2 scores indicating that the locations were most influenced by seasonality with respect to inorganic nutrients and biomass (Figure 8b). *P. antarctica* abundance was higher during December of 2001 and 2003 than 2004. PC2 and PC3 scores were also plotted and PC2 has negative loadings of season and 19-hex (Appendix D). PC1 and PC2 were plotted for each year separately to determine if seasonal differences (December vs. February) affected the data set (Appendix E-G). PC2 separated out December and February for every year except 2004. This is probably due to the absence of *P.antarctica* that December.

In order to draw a conclusion on how temperature may affect sample observations for each station, PC1 versus PC3 scores were plotted for the December stations (Figure 9b). December 2004 stations dominated by diatoms exhibited the highest scores, while in December 2001 and 2003 stations dominated by *P. antarctica* exhibited high negative scores. This suggests that temperature may play a role in assemblage composition and inorganic nutrient uptake. This is further confirmed with a significant relationship between temperature and percentage of prymnesiophytes for all December stations (Table 8). The percent prymnesiophyte data were collected from the IVARS CHEMTAX dataset located at http://www.vims.edu/bio/ivars. This suggests that lower temperatures favor prymnesiophytes (*P. antarctica*) while higher temperatures favor other phytoplankton including diatoms (Table 8, R^2=0.702, $p<0.0001$).

Temperature vs nitrate concentrations

Relationships between surface concentrations of nitrate and surface seawater temperature were examined to address the role temperature could play in nitrate uptake during December. There was a significant negative relationship between nitrate with

temperature, suggesting that temperature does play a significant role in Ross Sea nutrient dynamics. In order to confirm that this relationship has existed in historical data sets for the Ross Sea, the U.S. Joint Global Ocean Flux Study from the Southern Ocean Ross Sea cruises were analyzed for this relationship (http://usjgofs.whoi.edu/jg/info/jgofs/southern /nbp97_8/). There were 58 samples taken during the 97-8 cruise (late November to Mid December) for which measurements of nitrate and temperature data from the surface (0-5 m) were available. These data were combined with the IVARS data and regression analysis found a significant relationship between nitrate and temperature (Table 8, R^2= 0.756, $p<0.0001$).

Surface nitrate and temperature were found to have a significant relationship in our study. A model using the Goes et al. (1999) method with sea surface temperature and chlorophyll was developed to establish whether Ross Sea nitrate concentrations could be estimated by satellite measurements. A Ross Sea model for austral spring surface nitrate concentrations was developed using the following relationship:

$$\text{Nitrate} = 18.3 - 3.67\ \text{temp} - 0.571\ \text{temp}^2 + 0.351\ \text{Chl} - 0.0511\ \text{Chl}^2.$$

Based on this relationship, we can conclude that it may be possible for nitrate concentrations in austral spring to be predicted by satellite measurements. This is important as the timing of blooms and export could be approximated using only remote data. Our predicted values using this model compared to our actual shipboard measurements of nitrate were also significant (Table 8, R^2=0.834, $p<0.0001$).

DISCUSSION

Depth variation of inorganic nutrients and organic matter concentrations

We observed a relationship between depth of sample and inorganic nutrient concentrations using PCA analyses. Specifically, the relationship of nitrate concentrations to PC1, PC2, and PC3 are of interest due to the controls they could have on new production which defines the rate at which organic matter and energy may be passed onto higher trophic levels (Dugdale and Goering, 1967). Nitrogen concentrations and biogeochemistry of the Ross Sea are well established as the area has been intensively studied over the past 15 years (Smith and Anderson, 2003). However, interannual variations in nutrient uptake and phytoplankton dynamics are not well known though interannual variations in nutrient budgets have been recently published (Smith et al., 2006, section I).

Since irradiance decreases with depth, *in situ* nitrogen concentrations at the surface are lower due to increased biological uptake. While the relationship between irradiance and the uptake of nitrate by phytoplankton is poorly understood, Hu and Smith (1998) observed a strong dependence of nitrate uptake on irradiance using ^{15}N-labeled laboratory and field incubations of *P. antarctica* assemblages in the Ross Sea. Nelson and Smith (1986) and Glibert et al. (1982) suggested that the uptake of nitrate may be weakly dependent on irradiance and could be due more to species composition or intracellular reductants. Other studies in polar regions have found that the relationship between nitrate uptake and irradiance can depend on the growth stage of the phytoplankton bloom (Olson, 1980).

Seasonal variation

As expected, there were differences in concentrations of inorganic and organic matter between samples collected in December and February (Table 6). Many studies have suggested that phytoplankton growth in February may be limited by micronutrient concentrations (Sedwick et al., 2000; Peloquin, personal communication). There are also differences in the hydrographic parameters between the two seasons as indicated by the fact that the observations from December and February projected on different areas of the PC3 coordinate space (Figure 9b). While this study was primarily interested in how sea surface temperature may affect bloom growth in December, the PCA suggests that there are intensified seasonal variations in the Ross Sea, and that they are consistent interannually. The PCA also suggests that there are interannual variations in our study which, is confirmed by the variable year being strongly positively loaded on the PC2. Biogenic silica also is positively loaded on PC2 and shows seasonal and interannual variation as discussed by Smith et al. (2006) due to relative diatom abundance.

Relationship between temperature and nitrate uptake

The primary controls on the growth rates of Antarctic phytoplankton are still under debate (e.g., Arrigo et al., 1999; Smith et al., 2006). In this study we found a significant negative relationship between surface concentrations of nitrate and temperature (Table 4). Similar to Behrenfeld et al. (2006), this suggests that warmer temperatures in the Antarctic could affect nitrate uptake and species composition. Temperature was strongly related to PC3 and sites with warmer temperatures in December 2004 projected PC3 in the positive regions of PC3 coordinate space, while samples at colder stations in December 2001 and 2003 projected in the negative regions

(Figure 9b). Temperature, nitrate, and chlorophyll a concentrations have been linked due to incident radiation playing a role as a major heat source along with providing light for primary production and nutrient uptake (Kamykowsi, 1987). Smith and Harrison (1991) found that nitrate and ammonium uptake was directly affected by temperature and derived Q_{10} values were greater than 2. This same Q_{10} value has also been observed temperature/photosynthesis responses (Li et al., 1984). New production models including the relationship between nitrate and temperature have been applied successfully mainly due to rapid phytoplankton growth not being thought to diminish regenerated forms of nitrogen (Dugdale et al., 1989). There may be temperature dependence on iron uptake that is affecting nitrate uptake; however, the exact mechanism for the temperature dependence on iron uptake in marine algae remains undefined (Reay et al., 2001). In addition, temperature could be directly related to water mass intrusions which could have also brought iron into the surface waters (Peloquin, 2005).

Hydrographic properties exhibited interannual variability during austral spring (Table 5). Sea surface temperature was significantly higher in December 2004 than December 2001 and 2003. While an extensive analysis of sea ice, circulation, and irradiance is still being performed, temperature-salinity diagrams show that there is a high temperature/salinity signal below and above the pycnocline in December 2004, suggesting that these higher surface temperatures could be due to MCDW intrusions (Figure 12). Other regions in the Southern Ocean have been observed to have a limitation of nutrient drawdown and net productivity due to low temperatures (Tilzer et al., 1986; Priscu et al., 1989; Reay et al., 2001). It is unknown whether the phytoplankton assemblage is responding to the increase in temperature or other factors

since temperature can be a proxy for other parameters such as water mass intrusions that have higher concentrations of micronutrients (Grotti et al., 2001; Ianni et al., 2002). The presence of MCDW and AASW in the surface water has been attributed to increases in local nutrient and micronutrient concentrations through the melting of sea ice. These nutrient and micronutrient pulses affect local primary productivity and cause onsets of intense phytoplankton blooms. Future analysis will be performed on the relative roles of water mass intrusions and other factors such as irradiance and ice cover (Peloquin, unpublished data). Smith et al. (2006) noted large differences in ice cover between 2001 and 2003, and these could help explain the variability we are seeing in our data set as sea ice plays a crucial role in the quantitative and qualitative distributions of phytoplankton communities (Mangoni et al., 2004). Low water temperatures (< 0°C) have been reported to decrease phytoplankton production in other studies (Bracher et al., 1999; Saggiomo et al., 2002). Our study area was also affected by icebergs and high concentrations of sea ice, especially in December 2003 when ice concentrations were larger than the long term mean (Comiso et al., 1993; Smith et al., 2006). It should also be noted that while the nitrate uptake in December 2003 was extremely low, uptake during the growing season continued at elevated rates. As a result, the austral spring uptake represented only 31% of the seasonal removal due to a large bloom of diatoms later in the season (Smith et al., 2006). The phytoplankton assemblage during December 2003 exhibited stress both through a low P_m^B and F_v/F_m (Section IV; Peloquin 2005), and it is thought that the bloom of diatoms that followed was due to water mass intrusions that delivered iron to the surface waters (Peloquin and Smith, 1996).

Temperature and assemblage composition

Prymnesiophyte abundance was inversely related to warmer sea surface temperatures during our study (Table 8). *P. antarctica* has higher growth rates between -2 °C and 2 °C (Schoemann et al., 2005); therefore the warmer temperatures during December 2004 could have allowed diatoms to outcompete *P. antarctica*. *In situ* measurements comparing the growth rates of *P. antarctica* and diatoms solely due to temperature have not been published to our knowledge. In December 2004, diatoms were most abundant and this could be due to water mass intrusions like the ones observed for the secondary diatom bloom in austral summer of 2004 by Peloquin and Smith (2006). As mentioned, we also see in the T-S plots that warmer water was observed in the surface waters and at depth (Figure 10). While the secondary bloom during February 2004 was apparently fueled by MCDW intrusions during the austral spring, the T-S plots in December 2004 the following season show the significantly higher surface temperatures than the other study years. It is probable that along with MCDW, surface warming and ice melting caused additional decreases in salinity and increased warming during December 2004 which, are characteristic of Antarctic Surface Water (AASW) intrusions in the Ross Sea (Gordon et al., 2000; Grotti et al. 2001). High temperature, lower salinities (mean temperature and salinity: 1.0 °C, 34.19 in 1987/1988; 0.5 °C and 33.58 in 1989/1990), similar to December 2004 data have also been observed in the western Ross Sea and Terra Nova Bay and have been attributed to AASW (Grotti et al., 2001; and references therein). If these water mass intrusions, in fact, had increased iron concentrations, diatoms may be responding more quickly to these pulses of micronutrients.

Hydrographic properties including temperature clearly play a role in phytoplankton dynamics during our study and affect phytoplankton community composition. *Phaeocystis antarctica* was most abundant in December 2001 and December 2003 in the solitary and colonial form, respectively. While the life cycle and physiology of *P. antarctica* is poorly known, we observed a larger abundance of colonies in December of 2003. Section IV discusses the roles that various factors have on the dominant morphology of *P. antarctica* during our study. The larger percentage of diatoms during December 2004 may result from increased temperatures and micronutrients from the AASW and MCDW intrusions. The large secondary bloom in February 2004 has also been attributed to increased presence of MCDW (Peloquin and Smith, 2006). Bloom assemblage composition can also affect nitrogen uptake. Diatoms have been observed to remove both nitrate and ammonium more rapidly than *P. antarctica* (Smith and van Hilst, 2003)

Using remotely sensed data to predict nitrate concentrations

The Ross Sea nitrate model we generated could be used to generate large scale sea surface nitrate maps using remotely sensed temperature and chlorophyll data (Table 8). This model simply utilizes *in situ* chlorophyll and temperature from IVARS to see if it is possible to predict surface water nitrate concentrations in the Ross Sea using a simple mathematical relationship (see Methods section). Other regions have been successfully mapped nitrate with remotely sensed sea surface temperature values using similar models but are complicated models that include estimates of new production and utilize nitrate uptake rates and kinetics in California (Dugdale et al., 1997). Significant relationships between nitrate and temperature in CA (Kamkowski and Zentara, 2003), East China Sea

(Gong et al., 1995), and Eastern Tropical Pacific (Chavez et al,. 1996) have also been observed. Steeper slopes (relationship between temperature and nitrate) are seen at high latitudes, while shallower slopes are observed at lower latitudes (Zentara and Kamkowski, 1977). This implies that phytoplankton at higher latitudes respond to increases in temperature, with respect to nitrate uptake, at a faster rate than lower latitude phytoplankton.

Conclusions

The results from the PCA suggest that temperature may be one of the primary controls on austral spring bloom nitrate uptake dynamics, and this supports other studies arguing that *P. antarctica* has a dramatically reduced nitrate assimilation capacity in the Southern Ocean. Currently, it is not clear whether phytoplankton are responding to temperature or if it is a proxy for other parameters that can affect phytoplankton growth rates such as increased concentrations of micronutrients (related to the water mass intrusions) that were not measured in our study. Furthermore, analysis of the chlorophyll, temperature, and nitrate data from IVARS showed that utilizing satellite observations of temperature and chlorophyll as an estimate of new production during austral spring should be further explored. While the inverse relationship between water temperature and nitrate concentration is well established, this is the first study showing that this relationship holds true in the Ross Sea and affects phytoplankton community composition. Since temperature may be having negative effects on nitrate uptake by phytoplankton, enhanced iron may not fully allow the phytoplankton to deplete the nitrate pool due to low temperatures inhibiting nitrate and nitrite reductase. Temperature-salinity diagrams show that there is a high temperature/salinity signal below and above the

pycnocline suggesting that these higher surface temperatures in 2004 could be due to MCDW or AASW intrusions. Future studies looking at the magnitude and timing of these water mass intrusions and the effects on local temperature and micronutrient concentrations are essential in understanding phytoplankton dynamics. Finally, additional studies looking at the thermal behavior of enzymes in nitrate reduction and the capacity of phytoplankton to adapt to polar conditions are needed to fully understand the role that temperature plays in controlling austral spring bloom formation in the Ross Sea.

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Table 5. Means and standard deviations for mixed layer (Z_{mix}), surface temperature ($T_{surface}$), surface salinity ($S_{surface}$), and density ($\sigma_{t\ surface}$) for December 2001, 2003, and 2004. Bold numbers indicate a significant difference between other years.

Year	n	Z_{mix} (m)	$T_{surface}$ (°C)	$S_{surface}$ (psu)	$\sigma_{t\ surface}$
2001	5	23.5 ± 8.8	-0.96 ± 0.17	34.44 ± 0.09	27.70 ± 0.07
2003	6	19.1 ± 8.6	-0.90 ± 0.33	**34.14 ± 0.08**	**27.45 ± 0.06**
2004	20	21.7 ± 6.5	**0.14 ± 0.60**	34.44 ± 0.07	27.65 ± 0.04

Table 6. Means and standard deviations of sea surface nutrient and pigment concentrations in December of 2001, 2003, and 2004. Bold numbers indicate a significant difference between other years.

Year	n	NO_3 (μM)	$Si(OH)_4$ (μM)	PO_4 (μM)	Chlorophyll (μg L^{-1})	Fucoxanthin (μg L^{-1})	19-hex (μg L^{-1})
2001	5	22.04±1.28	**77.16±1.68**	**1.82±0.06**	5.81±1.91	1.82±0.63	9.29±2.63
2003	6	20.04±1.68	66.58±4.75	**1.58±0.087**	5.35±3.51	1.45±0.62	4.04±3.06
2004	17	**16.52±2.98**	63.54±8.65	**1.08±0.14**	7.90±3.94	3.78±3.42	**0.32±0.28**

Table 7. Variable loadings for principal components 1, 2, and 3.

Symbol	Variable	PC1	PC2	PC3
Year	Year	0.002	0.15	0.379
Season	Season	0.05	-0.449	0.198
Longitude	Longitude	0.021	-0.091	0.168
Z	Depth	-0.198	-0.129	-0.124
N	Nitrate	-0.239	-0.222	-0.225
P	Phosphate	-0.157	-0.189	-0.276
S	Silicic Acid	-0.23	-0.286	0.072
Tchl	Total Chlorophyll *a*	0.333	-0.164	-0.132
5ch	>5 μm Chlorophyll *a*	0.332	-0.174	-0.004
20chl	>20 μm Chlorophyll *a*	0.336	-0.113	0.002
<20chl	<20 μm Chlorophyll *a*	0.171	-0.175	-0.299
BSi	Biogenic Silica	0.319	0.129	0.017
POC	Particulate Organic Carbon	0.313	-0.224	0.07
PN	Particulate Nitrogen	0.334	-0.147	0.071
fuco	fucoxanthin	0.298	-0.029	0
hex	19'-hexanoyloxyfucoxanthin	0.021	-0.369	-0.168
temp	Temperature	0.095	0.176	0.47
sal	Salinity	-0.147	-0.314	0.41
dens	σ_T	-0.172	-0.359	0.326

Table 8: Regression results from comparisons of IVARS data with JGOFS and modeled data. See text for full description of analyses.

Parameters	R^2	p
Temperature vs. percentage of haptophytes	0.702	<0.0001
Temperature vs. *in situ* nitrate concentration	0.756	<0.0001
Modeled nitrate concentration vs. IVARS *in situ* nitrate concentration	0.834	<0.0001

Figure 6. Ross Sea station locations that were included in principal components analysis. (Courtesy of Jessica Walker, Raytheon Polar Services)

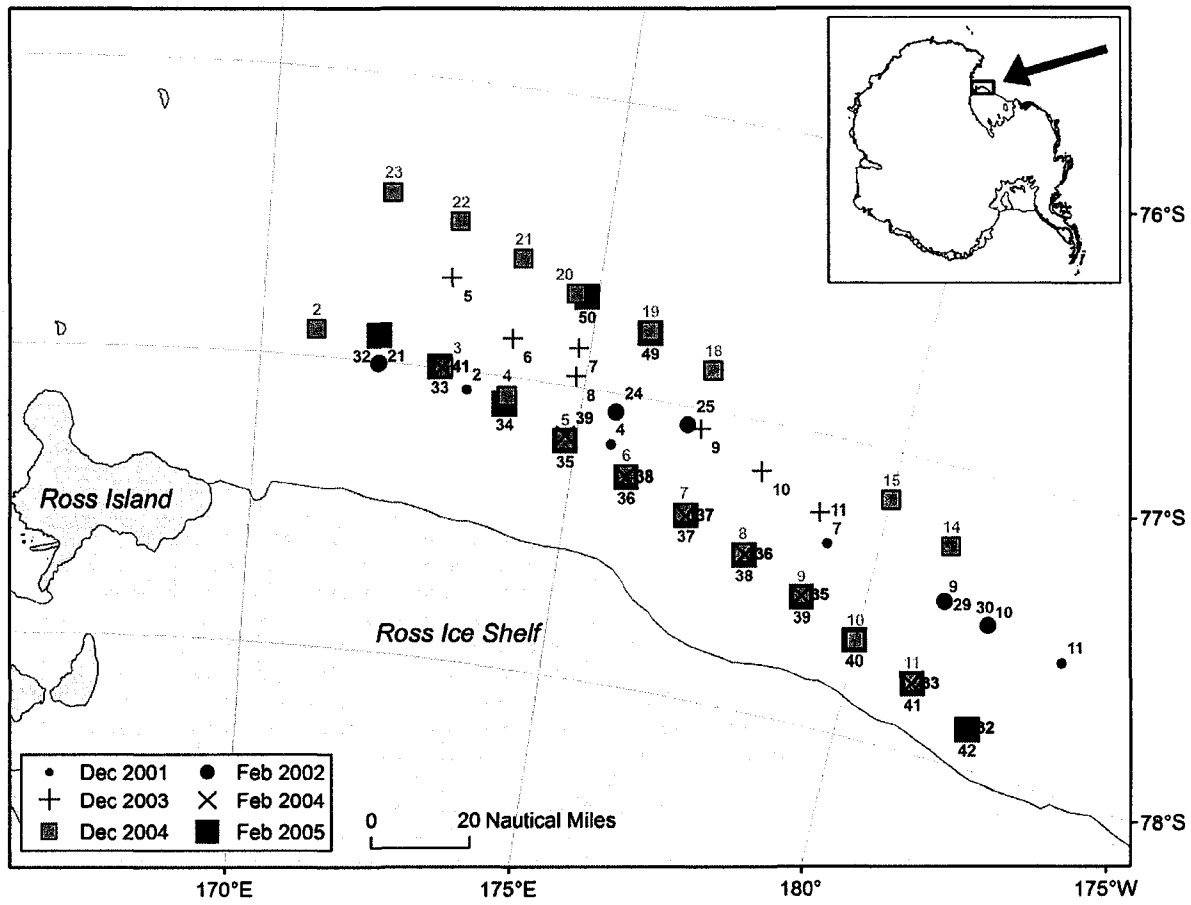


Figure 7. Phytoplankton community composition calculated from cell counts for IVARS December 2001, 2003, and 2004 (Peloquin et al. in prep).

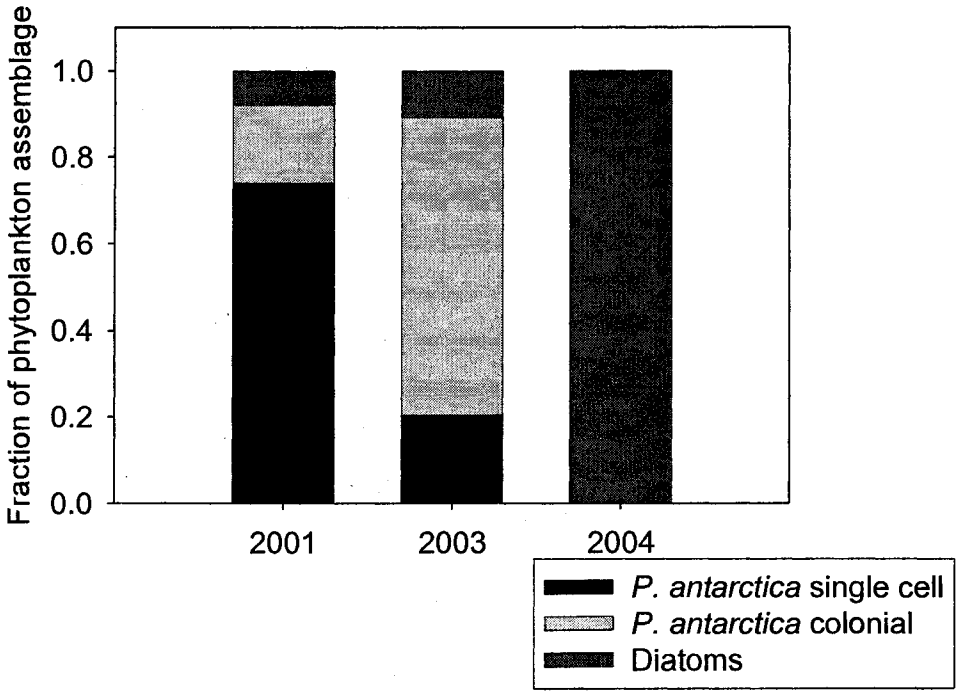
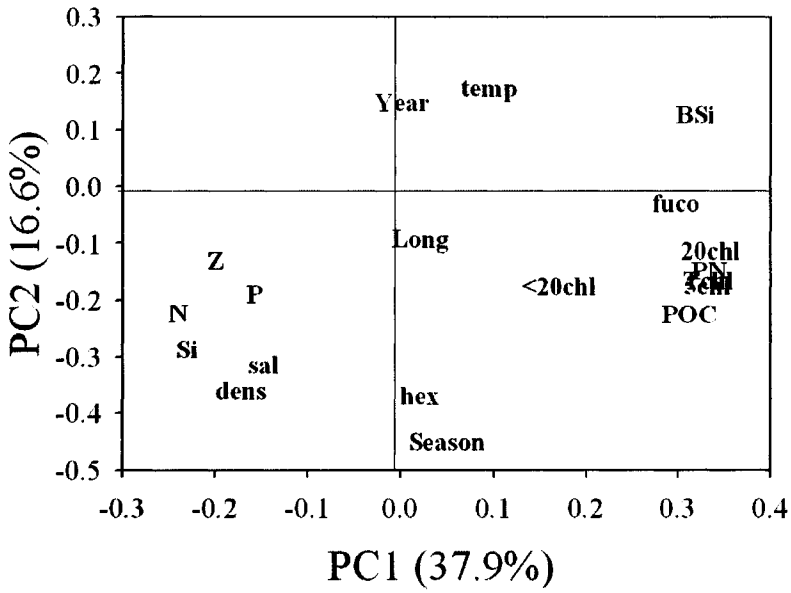


Figure 8. a) Principal components 1 and 2 loadings for IVARS data set. b) Principal components 1 and 2 scores for IVARS data set. (Circles, triangles, and x symbols represent samples taken at 0, 20, and 50 m, respectively).



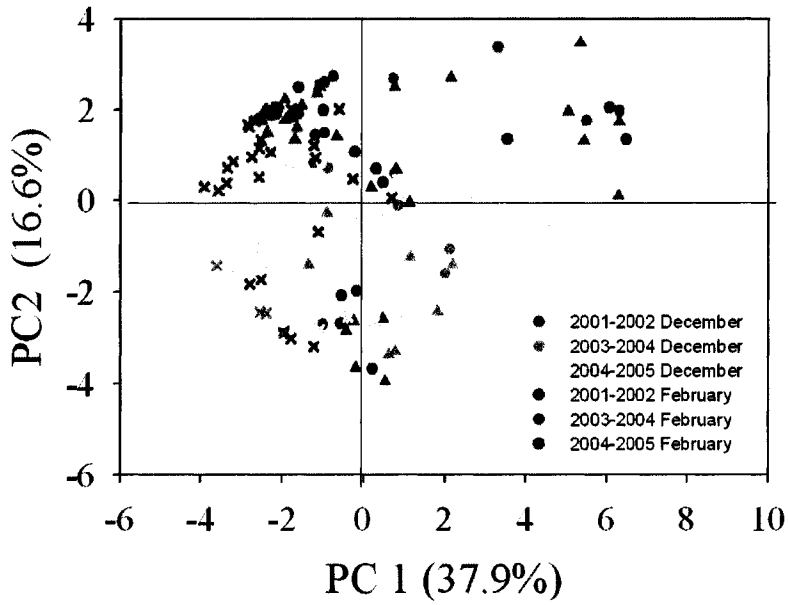


Figure 9 a) Principal components 1 and 3 loadings for IVARS data set. b) Principal components 1 and 3 scores for December data for IVARS data set. December PC3 scores plotted alone show separation of stations by year with the warmest years in 2004. (Circles, triangles, and x symbols represent samples taken at 0, 20, and 50 m, respectively).

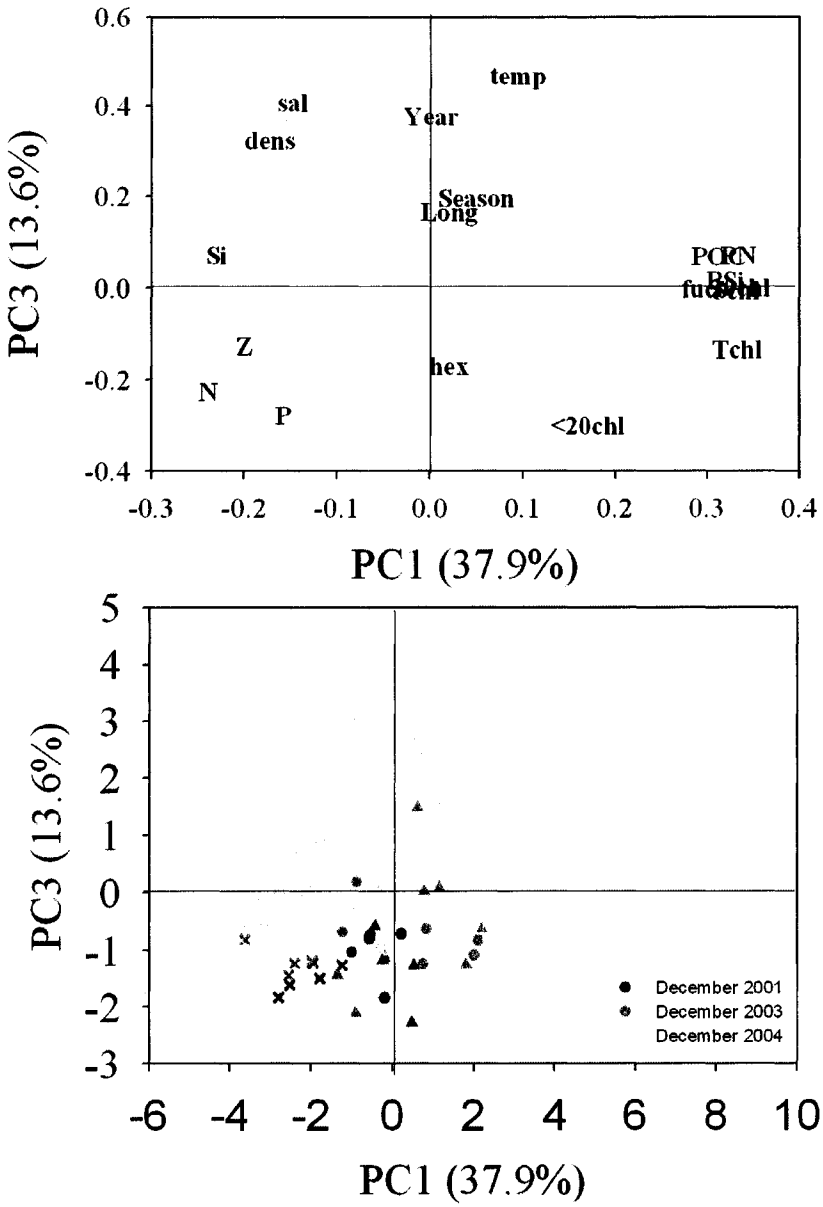
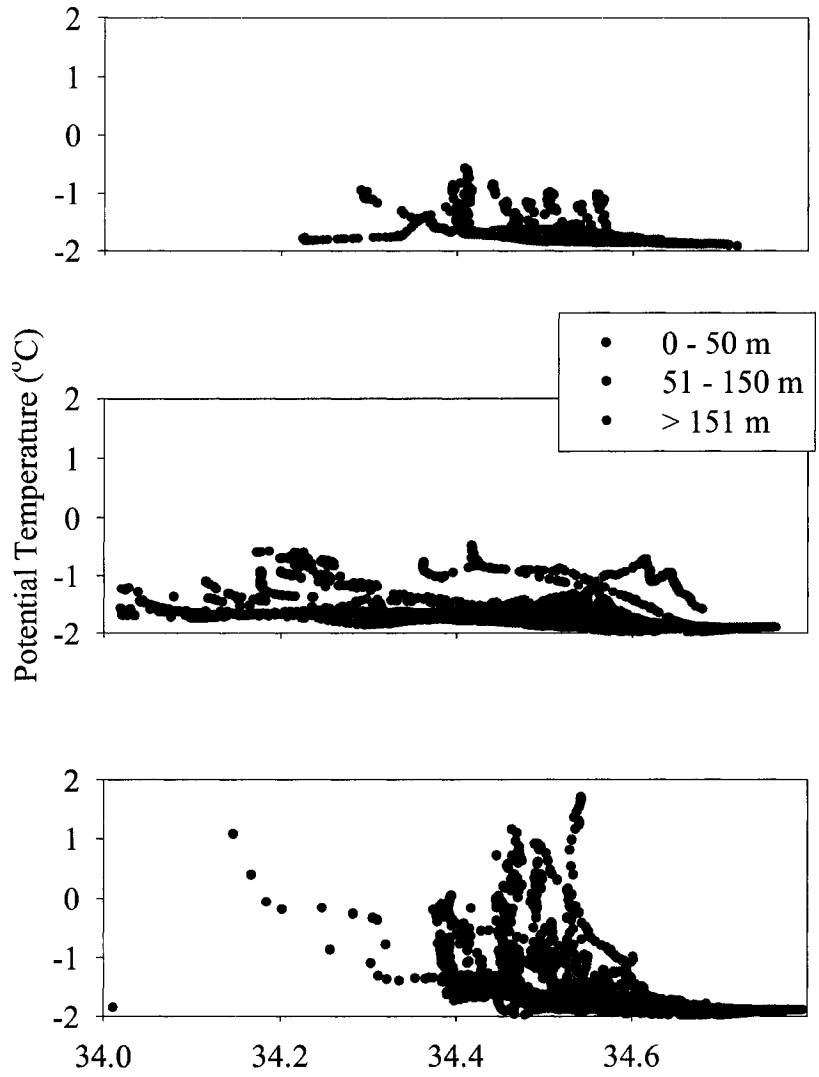


Figure 10. Potential temperature/salinity diagram for December 2001, 2003, and 2004. Potential temperature removes the effect of compressibility.



Section III. Interannual variability in vertical particle flux in the Ross Sea, Antarctica

ABSTRACT

The Ross Sea has one of the most spatially intense and predictable phytoplankton blooms in the Southern Ocean. The phytoplankton blooms differ in size and composition (diatom-and *Phaeocystis*-dominated blooms) which, have different impacts on carbon cycling due to the relative contribution of passive sinking and grazing by zooplankton. The main objectives of this study were to measure interannual variability in total mass flux (TMF), particulate organic carbon (POC), particulate nitrogen (PN), and biogenic silica (BSi) fluxes in the Ross Sea as part of the Interannual Variations in the Ross Sea-Antarctic (IVARS) program. Two sediment traps were deployed during 2003-2004 and 2004-2005 with one trap located in each of the southwestern (*Callinectes*) and southeastern (*Xiphias*) sectors. Variations in fecal pellet contribution to carbon flux, and the color and shape of the pellets were also examined. During our study phytoplankton community structure differed between 2003-2004 and 2004-2005 both assemblage composition and magnitude of biomass (Section I and II). The largest fluxes intercepted during our mooring deployments were in 2003-2004. During 2003-2004 the trap located in the southeastern region (*Xiphias*) had the highest TMF, BSi, and OC flux; this is unexpected, as in previous years the southeastern region has been observed to have lower concentrations of diatoms than the southwestern region. This increase in flux in the southeastern region is due to the large secondary diatom bloom in late January of 2004, with chlorophyll and POC greater or equal to the December blooms in 2003 and 2004. Herbivory by large zooplankton during 2003-2004 and 2004-2005 was also quite high, as confirmed by the abundance of green and brown pellets and the large percentage of

carbon flux represented by fecal pellets. Hence, interannual variation in grazing assemblages impacts export and biogeochemical cycles in the Ross Sea.

INTRODUCTION

Ross Sea vertical export

The Ross Sea has one of the most spatially intense and predictable phytoplankton blooms in the Southern Ocean (Sullivan et al., 1993; Nelson et al., 1996). The phytoplankton blooms differ in size and composition (diatom- and *Phaeocystis*-dominated blooms), resulting in different impacts on carbon cycling due to the relative contribution of passive sinking of phytoplankton aggregates and grazing by zooplankton (Dunbar et al., 1998; Accornero and Gowing, 2003). Smith and Dunbar (1998) and Asper and Smith (1999) found that the export of particulate organic carbon from the euphotic zone was temporally uncoupled to surface layer production and biomass. They also found that as the bloom of *Phaeocystis antarctica* reaches its maximum biomass, aggregates form which, significantly accelerate export to depth. The formation of aggregates is a function of particle stickiness, turbulence, cell abundance and the abundance of colonies. Generally, aggregates increase sinking rates from the euphotic zone (Wassman, 1994), although some *P. antarctica* cells may be released from sinking colonies and aggregates.

Supply of material from the overlying water column and regeneration of biogenic material in the water column affect the biogenic composition of the sea bed. The particulate matter composition at depth is dependent on biological factors such as primary and secondary production, chemical factors such as dissolution and oxidation, and physical factors such as aggregation, vertical and lateral transport (Lal and Lerman, 1975). A significant amount of biogenic matter is produced in the surface waters of the Ross Sea and subsequently accumulates in the sediments (Ledford-Hoffman et al., 1986; DeMaster et al., 1992). These vertical fluxes of biogenic silica and particulate organic

carbon are largely constrained to the continental shelf. The extent of lateral transport of particles is still unclear in the Ross Sea (DeMaster et al., 1992).

Zooplankton and vertical flux

The phytoplankton and zooplankton community affects the biological pump, which is the principal biological regulator of ocean-atmosphere carbon cycling. The biological pump removes carbon from the surface ocean to the deep ocean through the settling of particulate organic matter (Longhurst and Harrison, 1989). The organic matter in the surface waters either enters the microbial loop and is remineralized in the surface or it sinks out of the surface waters through passive sinking of aggregates, active grazing by zooplankton via sinking of fecal pellets, and active transport by zooplankton. Mesozooplankton (multicellular organisms that range from 200 μm to 2 mm in size) feed on phytoplankton, other mesozooplankton, and microzooplankton. The relative roles of microzooplankton (associated with regenerated production) and mesozooplankton (associated with export) grazing have a large impact on ecosystem function and the magnitude of vertical flux in marine systems on biogeochemical cycles. Micro- and mesograzing is thought to have little influence on vertical flux in the Ross Sea when colonies of *Phaeocystis antarctica* are present (Caron et al., 2000). Grazing on colonies and its effect on sinking rates due to fecal pellet production is complex and is affected by physiology and microbial colonization of the colonial matrix (Bautista et al., 1992). Since spring primary productivity is often dominated by *P. antarctica* (Smith and Gordon 1997), the export flux often consists of rapidly sinking colonies and aggregates. Herbivorous mesozooplankton have been observed to ingest *P. antarctica* (Shields, unpublished data). Diatoms, however, are large enough to be eaten by mesozooplankton

and macrozooplanton (>10 to >500 μm for diatom species forming large chains), and therefore can be exported in rapidly sinking fecal pellets (Bathmann et al., 1990). Diatoms also can sink as individual cells, spores or aggregates without being consumed (Smetacek, 1985; Alldredge and Gotchalk, 1989).

Zooplankton fecal pellets play an important role in transporting organic material to the deep ocean and nutrient cycling (e.g. Small et al., 1979; Turner and Ferrante, 1979; Steinberg et al., 2000; Turner, 2002; Schnack-Schiel and Ilsa, 2005). The sinking speed of the zooplankton fecal pellets is related to its size and therefore flux is related to the size structure of the grazer community (Small et al., 1979; Uye and Kaname, 1994; Turner, 2002). Potential sinking velocities in the Ross Sea range from 25 to 677 m d^{-1} (Accornero and Gowing, 2003). Density and size of fecal pellets from copepods are related to food availability and food type (Feinberg and Dam, 1998). Fragile, less compact fecal pellets produced by copepods with low food availability or quality are less likely to sink out of the euphotic zone than other pellets produced during high food concentrations (Dagg and Walser, 1986). Hydrodynamics, aggregation, bacterial decomposition, and destruction and ingestion by other zooplankton affect the sinking rate and preservation of fecal pellets (Honjo and Roman, 1978; Uye and Kaname 1994).

The use of sediment traps for measuring vertical flux can have significant errors associated with their deployments (Buessler et al., submitted). These include "swimmers" (Hedges et al., 1993; Honjo, 1996; Buessler, 1998; Buessler et al., submitted), the horizontal advection of particles (Buesseler, 1991), particle degradation and the efficiency of the trap (Buessler et al., submitted). Two recommendations to increase trap efficiency are to use neutral buoyant traps to avoid hydrodynamic issues and

to limit access of zooplankton to traps (Buessler et al., in press). Even with these errors, sediment traps can provide valuable information about the role of grazing in fecal pellet flux in the Ross Sea since traps allow for the direct collection and examination of sinking particles.

Biological mediation of particle flux in the Ross Sea is the main process investigated in this study. While there is a lag in the peak of accumulation of organic matter in the surface waters during austral summer, the timing and magnitude of fecal pellet flux during two years with different dominant phytoplankton assemblages provides an opportunity to determine how primary and secondary producers affect export and flux to depth in the Ross Sea. The main objectives of this study were to measure interannual variability in total mass flux (TMF), particulate organic carbon (POC), particulate nitrogen (PN), and biogenic silica (BSi) fluxes in the Ross Sea as part of the Interannual Variations in the Ross Sea-Antarctic (IVARS) program (Section I and II). Two traps were deployed in each of two years (2003-2004 and 2004-2005) with one trap each in southwestern (*Callinectes*) and southeastern (*Xiphias*) sectors. Variations in fecal pellet contribution to carbon flux, and the color and shape of the pellets were also examined. During our study the phytoplankton community composition between 2003-2004 and 2004-2005 differed in both species and concentration (Section I and II). Based on this observation, I hypothesized that *Phaeocystis*-dominated stations in late December of 2003 would have higher flux as aggregates and organic carbon export, and diatom-dominated sites later in austral summer (February 2004) and throughout the austral spring and summer of 2004-2005, would have silica and carbon fluxes dominated by fecal pellets. Grazing on *Phaeocystis* colonies is minimal, while mesozooplankton and

macrozooplankton grazing on diatoms in the Ross Sea is high. In the southwestern Ross Sea it was expected that our moorings would primarily consist of fecal pellets from zooplankton that grazed diatoms and diatom aggregates, while in the southeastern region I expected to see flux mainly from phytoplankton aggregates and higher ratios of carbon flux to opal flux.

METHODS

As a part of the Interannual Variability in the Antarctic- Ross Sea (IVARS) program, two McLane Mark 78-H PARFLUX sediment traps were deployed on moorings each year (2003-2004 and 2004-2005) along either end of our ship transect in December and retrieved in February (Tables 9, 10). The sediment traps (with 21 cups) were deployed along with a timer (rotating every two days; for a maximum of 38 days) on either end of the southernmost transects of the IVARS cruises with *Callinectes* located in the southwestern Ross Sea where primary productivity is usually attributed to diatoms (DeMaster et al., 1992; Smith et al. 1996). The *Xiphias* mooring was deployed in the southeastern region which, usually is dominated by *Phaeocystis antarctica.* These sediment traps were moored at Station 3 (*Callinectes*) and Station 10 (*Xiphias*) at 200 m (Figure 1 from Section 1). The traps were deployed along with water samplers, nitrate and silicate analyzers, thermographs, fluorometers, a MAV-3 current meter, and a Microcat CTD (Figure 11). The United States Coast Guard icebreaker *Polar Star* and RVIB *Nathaniel B. Palmer* were used for the deployment and retrieval of the sediment traps. Nineteen samples (two day intervals, 38 days) at each of the *Xiphias* and *Callinectes* locations were collected between December 31-February 2, 2004 and December 30, 2003 to February 4, 2004, respectively. During 2004-2005, seventeen

samples (two-day intervals, 34 days), were taken at *Xiphias* from December 25, 2004 to January 29, 2005. *Callinectes* was deployed from December 23, 2004 to January 29, 2005 (two-day intervals, 36 days). Before deployment, each sample cup was filled with preservative solution that consisted of buffered 2% formalin and 50 g L^{-1} NaCl final concentration. Strontium chloride (final concentration 10 mg L^{-1}) was added for the preservation of Acantharia. For detailed procedures, see Asper and Smith (1999).

Sample Processing

Swimmers, zooplankton that swim or are advected into the traps, were removed from the samples using a 600 μm mesh. Due to the large size of mesh, it is likely that there still were zooplankton remaining which, would cause an additional source of error. Each sediment trap sample was split into four samples using a four way plankton splitter for aliquots for TMF, BSi, POC/PN, and microscopy. Total mass flux (TMF or dry weight) samples were processed by preparing preweighed 0.8 μm poretic filters that were rinsed three times with distilled water and then were dried and reweighed. Samples were then filtered onto the preweighed filters and the filter funnels and filters were rinsed with ammonium formate to remove sea water salts. The filters were then dried at 60 °C for approximately four days, until a constant weight could be obtained. POC and PN samples were filtered onto precombusted GF/F filters and then rinsed with 5 mL 0.01N HCl to removed inorganic carbon. Filters were then placed onto a piece of combusted foil and dried in an oven at 60 °C for at least five days to ensure dryness. Once dry, POC/PN samples were run using the protocol in Section I (Asper and Smith, 1999). Biogenic silica (BSi) samples were filtered onto 0.6 μm polycarbonate filters and dried at 60 °C for 2-3 days and then run using protocol from Section I. Total particulate organic carbon, total

particulate nitrogen, biogenic silica and total mass flux (TMF) were determined by the following equation:

$$TMF = \text{Total mass per sample} / (\text{Time interval} * 0.5\ m^2).$$

Microscopy

Aliquots for microscopy were further split using a smaller two-way Folsom plankton splitter until approximately 100 fecal pellets per sample were left for enumeration. Samples were then concentrated using a 53 μm sieve; minipellets were not included in microscopic analyses. These samples were placed in a clean Petri dish and imaged using an Olympus stereo dissecting microscope. Images of fecal pellets for 4-5 samples of each trap for each year were analyzed using Image Pro software from which the length and width of each individual pellet in the trap material was measured. Observations were taken from 1774 pellets, including morphology and qualitative color. Fecal pellet carbon was measured using the volumes calculated from microscopy (Kelchner, 2005) and two volume to carbon values from the literature. Fecal pellet carbon values measured in the field were not available for the Ross Sea; therefore, the lowest literature value used for other studies in our region (0.016 mg C mm^{-2}, euphausiid pellets from Norway) and a middle range value (0.05 mg C mm^{-2}, copepod pellets from Norway) were used to show the error surrounding these measurements (Gowing et al., 2001 and references therein).

RESULTS

Biogeochemical properties

Flux during 2003-2004

Total mass flux, biogenic silica, organic carbon, and particulate nitrogen flux varied interannually, seasonally, and spatially (Tables 9, 10; Figure 12a-d). During 2003-2004 flux was higher at *Xiphias* with 1177 mg m^{-2} (TMF), 82.8 mg m^{-2} (BSi), 143.1 mg m^{-2} (POC) and 16.3 mg m^{-2} (PN) over the duration of the trap deployment (Table 9, 10). These values were 2-3 times higher than *Callinectes* (more western location). *Xiphias* also had two periods of high flux (the periods between January 6-10 and January 26-February 5) (Figure 12a). *Callinectes* only had one period of high TMF on January 5 (Table 9, Figure 12b). The secondary fluxes of BSi, TMF, and OC appear to be due to the large secondary diatom bloom that year (Section I).

Flux during 2004-2005

During 2004-2005 *Callinectes* had higher TMF, BSi, POC, and PN fluxes than *Xiphias* of 565.6, 53.6, 133.2, and 24.3 mg m^{-2} d^{-1}, respectively (Table 10). The highest TMF flux rate during 2004-2005 was on January 20th with a TMF of 58.31 mg m^{-2} d^{-1} (Figure 12d). TMF Flux during 2004-2005 for both trap locations was 2-3 times lower than 2003-2004 TMF flux at the *Xiphias* mooring (Figure 12c,d). The POC flux during 2004-2005 at both trap locations, however, was similar to *Xiphias* 2003-2004.

Relationship between TMF and composition

Regression analyses show that TMF has a significant relationship with BSi, POC, and PN for both years and trap locations (Table 11, R^2=0.38, $p<0.0001$; R^2=0.37, $p<0.0001$; R^2=0.13, $p=0.003$ for BSi, POC, and PN, respectively). POC and PN also had

a significant relationship throughout both years and trap locations (Figure 13, R^2=0.879 $p<0.0001$). The C/N ratio never exceeded 13.8 during 2003-2004 and 10. 7 in 2004-2005, and usually was much lower, closer to the Redfield ratio (Table 9-10).

Biogenic silica flux remained low during the periods of December 23 to mid-January for both locations for both years except *Xiphias* in 2004-2005 which, had maximal BSi fluxes of 4.2 mg m^{-2} d^{-1} on January 3 (mean ± sd: 1.1± 1.0) (Table 10). During 2003-2004 the December bloom was dominated by colonial *P. antarctica*, while in 2004-2005, diatoms were more abundant (Section II) which, may explain why biogenic silica fluxes were so low. The secondary diatom bloom in February 2004 (Section II) is reflected in the TMF, BSi, and POC fluxes of the deployment during the 2003-2004 growing season (Figure 12 a,b). While the bloom was observed at both *Xiphias* and *Callinectes*, the highest flux was at *Xiphias* in 2004. The secondary bloom during 2003-2004 therefore provided an additional BSi flux equal to the total BSi flux from the December phytoplankton bloom in 2004-2005, suggesting that the secondary blooms may significantly increase the amount biogenic fluxes. Biogenic silica also had a positive relationship to POC and PN fluxes; therefore, diatom blooms may have more of an effect on POC flux than *P. antarctica* or act as ballast material for aggregates (Table 11, R^2=0.45, $p<0.0001$; R^2=0.17, $p=0.0004$ for BSi vs. POC and PN, respectively).

Fecal Pellets

Fecal pellet carbon flux differed significantly interannually, seasonally, and spatially during 2003-2004 and 2004-2005 (Tables 9, 10; Figure 14). The highest fecal pellet organic flux during 2003-2004 was at *Xiphias* on February 5, 2004 and was 7.4 mg C m^{-2} d^{-1} or 111.1 % of the trap carbon flux. This was using the higher volume to carbon

conversion ratio used for ovoid fecal pellets which dominated the trap sample. Using the lower volume to carbon conversion factor, the fecal pellet carbon was only 2.4 mg C m^{-2} d^{-1} or 36.3% of the trap carbon flux (Table 9). During some periods of 2004-2005, fecal pellets also represented a high percentage of carbon flux, with the highest fecal carbon flux of 4.7 mg C m^{-2} d^{-1} or 38.5% of the carbon flux on January 3, 2005 (Table 10).

Ovoid pellets were the most observed fecal pellet shape in our study. Cylindrical, spherical, and rectangular pellets were also observed. The ovoid fecal pellet shape was similar to *Oithona* sp. pellets (Martens, 1978), however they were much larger in most cases, ranging from 200-300 μm in length and 100-200 μm, in width. *Oithona* sp. in other regions have a fecal pellet mean length and width of 36 and 26 μm (Martens, 1976). Visual observations of net tow materials suggest that *Oithona* sp. was not highly abundant at the end of January in 2005, while calanoid copepods, Acantharia, euphausiids, and colonial choanoflagellates were dominant (Shields, unpublished). Fecal pellet color also varied widely between different stations and over time (Figure 15a-c). While no relationships or correlations can be made with these data, fecal pellets that were lightly colored (Pellet D, Figure 15a) were associated with all the large carbon fluxes, while TMF green or F (Figure 15b, pellet F) pellets were more associated with TMF. The length and width measurements of the pellets intercepted by each trap cup are given in Appendix H and I. Forams and other components including diatom fluff and amorphous aggregates were also observed in addition to the pellets.

DISCUSSION

Variation in flux

These results provide insights about fluxes during austral summer and their relationship to phytoplankton assemblage composition. Flux is usually highest from mid-January to early March in the Ross Sea (Dunbar et al., 1998; Sweeney et al., 2000; Kelchner, 2005). The largest fluxes intercepted during our mooring deployments were in 2003-2004 (Table 9). During 2003-2004 the southeastern trap (*Xiphias*) had the highest TMF, BSi, and POC flux. This was unexpected as in previous studies the southeastern region has been observed to have lower concentrations of diatoms than the southwestern region. This increase in flux in the southeastern region is due to the large secondary diatom bloom in late January of 2004 with chlorophyll and POC concentrations greater or equal to the December blooms in 2003 and 2004. The chlorophyll concentrations were an order of magnitude higher in February 2004 than February 2005. This secondary diatom bloom might have provided sufficient ballast for *P. antarctica* aggregates, making the *P. antarctica* aggregates more dense and sinking faster out of the water column. This bloom also provided food for zooplankton as fecal pellets represented 111.1% of the OC at *Xiphias* and 35% at *Callinectes* at the end of the season (Table 9, 10). The low fecal pellet flux during early January 2004 could be due to remineralization of *P. antarctica* blooms since the removal of carbon through heterotrophic mineralization within the water column is significant in the Ross Sea (Smith and Dunbar, 1998). The high percentage of fecal pellet carbon flux (>100%) could be due to the higher volume to carbon conversion used in this study. Additional work is needed to get accurate volume to carbon conversions for zooplankton fecal pellets in the Ross Sea.

The C/N ratio of the organic matter was usually close to the Redfield ratio of 6.6 and never exceeded 13.8 during both years (Tables 9, 10). This suggests that food was abundant for herbivores, and the zooplankton pellets likely contained unassimilated organic matter since the C/N ratios approached the Redfield Ratio of 6.6 for living plankton (Knauer et al., 1979). The average C/N ratio for December 2003 when *P. antarctica* colonies were most abundant was 7.5 (http://www.vims.edu/ivars). C/N ratios of sediment trap matter ranging from 6-10 could reflect superfluous feeding (Knauer et al., 1979). Higher ratios could also suggest that nitrogen was preferentially removed through bacterial remineralization (Parsons et al., 1984).

Previous sediment trap studies measured higher POC fluxes with values up to 92.7 mg C m^{-2} d^{-1} for the same region and season (Smith and Dunbar, 1998; Fabiano et al., 1997; Pusceddu et al., 1999). Smith and Dunbar (1998) also observed a large percentage of fecal pellets in some of their western Ross Sea samples. Accornero et al. (2003) present a summary of OC, TMF, and BSi estimates by many researchers during January and February that are similar to our results (e.g. Dunbar et al., 1998; Accornero et al., 1999; Langone et al., 2000). Organic carbon fluxes ranged from 0.2- 180.3 mg m^{-2} d^{-1} during February 1990 to 1992 (Dunbar et al., 1998). TMF flux ranged from 0.4- 83.4 mg m^{-2} d^{-1} January 1995 to January 1996 (Accornero et al., 1999) and BSi ranged 0-64 mg m^{-2} d^{-1} from December 1994 to January 1996 (Langone et al., 2000).

Biogenic silica had a positive, linear relationship with POC flux (Table 11). Nelson et al. (1996) also found a similar relationship in the Ross Sea. Ballast minerals, including silicate and carbonate minerals, often comprise more than half of the mass of particles leaving the surface (Honjo, 1980, 1996) which, in turn allow particles to sink faster and

affect the exposure time of organic matter remineralization (Lee et al., 2004). Armstrong et al. (2002) noted that flux of organic matter at depths >1800 m did show a direct relationship to fluxes of ballast minerals but they did not find this at shallower depths. In the Ross Sea, however, we found this relationship as shallow as 200 m, suggesting that diatoms do play a crucial role in flux. Ballast minerals provide a way to protect organic matter though internal protection (organic matter incorporated into tests) so that it is not exposed to decomposing enzymes (Armstrong et al., 2002). The organic matter may also help bind particles together. Ballast also increases fluxes, leading to less time for water column remineralization in surface waters.

Carbon production and vertical flux

In the Ross Sea, there is a temporal offset between biomass and vertical export, with the highest flux reported later in the season than in other studies (Kelchner, 2005). Particulate organic carbon flux is uncoupled with surface primary production. The C/N ratios close to Redfield (6.8 in the present study) suggest that the material derived from phytoplankton and also could represent superfluous feeding during both seasons (Tables 9,10). Bacterial activity is reduced in polar regions (e.g., Karl, 1993) which may explain why we are getting significant relationships between carbon and nitrogen even at 200 meters.

Zooplankton Fecal Pellets

Fecal pellet flux varied between the two seasons measured (2003-2004 and 2004-2005) (Table 9, 10). There was a delay in the production and export in fecal pellets until mid-January and this may be due to the delay in development of the zooplankton grazing community (Dunbar et al., 1998; Sweeney et al., 2000). The production of pellets in the

Ross Sea appears to be by a zooplankton community that produces ovoid fecal pellets. These pellets were similar to those observed by Kelchner (2005), Accornero and Gowing (2003) and Honjo and Roman (1978). There was only one zooplankton tow performed during 2004-2005, and calanoid copepods were dominant. A detailed, quantitative analysis has not been performed. Other studies observed that amphipods dominated the Ross Sea surface zooplankton community (Pakhomov and McQuaid, 1996). Pteropods were also in high abundance during our tow in 2004-2005, and they are a common primary consumer in the Ross Sea (Seibel and Dierssen, 2003). Tabular salp and cylindrical Antarctic krill (*Euphausia suberba*) pellets are observed in other areas of the Ross Sea and the Southern Ocean (Biggs, 1982; Kelchner, 2005).

While fecal pellet size and morphology is indicative of different zooplankton taxa (Silver and Bruland, 1981), fecal pellet color can indicate food source and mode of feeding. The majority of the pellets intercepted in the traps were green and brown ovoid pellets (Figure 15a-c). Honjo (1978) and Honjo and Roman (1978) collected 'green' fecal pellets and observed phytoplankton pigments, coccoliths, and diatom fragments in green pellets. These green pellets contained only a small amount of organic material. The darker, well consolidated pellets that were similar in form contained more amorphous organic material with little diatom tests (Honjo and Roman, 1978; Wilson, personal communication). The high abundance of green and brown pellets in our study indicates that herbivorous grazing by large zooplankton occurred during 2003-2004 and 2004-2005. Fabiono et al. (1997) observed the same oval fecal pellets which, also dominated the fecal pellets their traps intercepted in Terra Nova Bay and were of unknown origin. Scanning electron microscopy of these pellets showed that these oval shaped fecal pellets

were made of densely packed diatom cells (Fabiano et al., 1997). Food quality also can affect fecal pellet volume, density, and carbon content of fecal pellets, suggesting that diatom and *Phaeocystis antarctica* blooms should have different impacts on carbon flux (Butler and Dam, 1994). Copepod fecal pellet production has been found to decrease up to 80% due to *Phaeocystis* sp. blooms in the North Sea Southern Bight (Frangoulis et al., 2001).

While pellets less than 100 μm were not considered in this study, "minipellets" can be a significant portion of the total flux in some regions (Gowing and Silver, 1985). Pteropods were present during our study and may also have contributed to some of the smaller fecal pellets less than 63 μm. However, the morphology of the pteropod, *Limacina limacina*, pellets and pteropod grazing on phytoplankton in the Ross Sea is unknown. Pteropod pellets could also be a source of high silica flux. Other pteropods, such as *Corolla spectabilis,* are associated with high silica flux in the central California current (Silver and Bruland, 1981).

Other Loss Processes

Physical forces may also affect flux in the Ross Sea. While a one-dimensional view is useful in understanding export, lateral advection in the Ross Sea may play an important role in decoupling surface waters with the underlying seabed (Leventer and Dunbar, 1987). Horizontal advection could also play an important role in the disjunction between production and vertical flux in the region. Currents could move particles up to 207 km over an 80-d period (period noted in Smith and Dunbar, 1998). While we did see interannual variability between *Xiphias* and *Callinectes* between 2003-2004, it is certain that the relationships between production, flux, and sinking rates of different material are

extremely complex. Carbon from the surface can leave through biological processes such as vertical migration, in which active transport by zooplankton bypasses sediment traps (Steinberg et al., 2000). Active transport ranged from 7.8- 38.6% of mean sinking POC flux at 150 m (Steinberg et al., 2000). Life history strategies of algae, protozoans and small metazoans that include vertical movements throughout the water column may average at least 35% of the carbon leaving the water column (range of 11-80%, does not include larger organisms) (Silver and Gowing, 1999).

Conclusions

The Southern Ocean is a significant part of the global carbon budget. In order to understand and predict particulate organic carbon flux in the Ross Sea, processes such as herbivorous zooplankton grazing rates and the role of food quality and quantity on carbon flux , must be explored further. Secondary blooms during February of 2004 caused significant pulses of organic matter to depth in the Ross Sea. As scientists begin to understand the role that modified water mass intrusions may have on these large secondary blooms in the Ross Sea, the increased pulses carbon flux and the resultant affects on the benthos could be significant. The high abundance of ovoid brown and green pellets in our trap samples suggests that herbivorous grazers in the Ross Sea may be important in carbon flux. Massive sinking of ungrazed *P. antarctica* blooms was not observed. Copepods may also be able to utilize *P. antarctica* as a food source and this could change our view of how the biological pump works during these large blooms.

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Table 9. Total mass flux, biogenic silica, particulate organic carbon, particulate nitrogen, and fecal pellet flux for trap deployed for December 2003-February 2004. Fecal pellet flux only measured for 4-6 samples per sediment trap deployment due to sample processing time. * Range of values using a low and intermediate volume to carbon literature conversion.

SAMPLE	Date	TMF	BSi flux		OC flux		N flux		Bsi/OC	C/N	Fecal Pellets
		mg m^{-2} d^{-1}	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	w/w	w/w	mg C m^{-2} d^{-1} *
XIPHIAS 1	12/31/2003	8.7	0.6	7.3	nd	nd	nd	nd	nd	nd	0.67-2.1
XIPHIAS 2	1/2/2004	12.5	1.2	9.4	1.9	15.0	0.2	1.4	0.6	10.5	
XIPHIAS 3	1/4/2004	11.3	1.2	11.0	3.4	30.2	0.4	3.5	0.4	8.7	
XIPHIAS 4	1/6/2004	70.2	0.9	1.2	4.3	6.1	0.4	0.6	0.2	11.0	0.26-0.80
XIPHIAS 5	1/8/2004	10.5	0.6	5.5	1.3	12.3	0.1	1.2	0.4	10.0	
XIPHIAS 6	1/10/2004	67.7	0.9	1.3	1.4	2.0	0.1	0.2	0.7	9.7	0.22-0.68
XIPHIAS 7	1/12/2004	9.7	0.6	6.0	1.9	19.9	0.2	2.3	0.3	8.5	
XIPHIAS 8	1/14/2004	6.9	1.1	15.8	3.5	50.2	0.4	5.7	0.3	8.8	
XIPHIAS 9	1/16/2004	4.7	0.6	12.1	1.8	39.3	0.2	5.3	0.3	7.4	
XIPHIAS 10	1/18/2004	26.8	0.7	2.7	1.8	6.9	0.3	1.0	0.4	6.8	
XIPHIAS 11	1/20/2004	22.5	1.1	4.7	2.5	10.9	0.4	1.6	0.4	6.7	
XIPHIAS 12	1/22/2004	27.1	1.2	4.4	2.9	10.8	0.4	1.5	0.4	7.1	
XIPHIAS 13	1/24/2004	51.5	4.6	9.0	8.9	17.2	1.0	2.0	*0.5*	8.8	0.67-2.1
XIPHIAS 14	1/26/2004	61.3	1.9	3.1	6.2	10.1	0.6	0.9	0.3	10.8	
XIPHIAS 15	1/28/2004	14.4	1.5	10.1	8.6	59.4	0.6	4.3	0.2	13.8	
XIPHIAS 16	1/30/2004	39.0	4.3	11.1	5.4	13.9	0.7	1.9	0.8	7.3	
XIPHIAS 17	2/1/2004	32.0	2.9	9.1	3.9	12.1	0.5	1.6	0.8	7.6	
XIPHIAS 18	2/3/2004	55.4	7.8	14.1	5.4	9.7	0.6	1.1	1.5	8.6	
XIPHIAS 19	2/5/2004	56.5	7.8	13.7	6.6	11.6	0.9	1.6	1.2	7.1	2.4-7.4
Total Flux		1177.3	82.8		143.1		16.3				

Table 9., ctd. Total mass flux, biogenic silica, particulate organic carbon, particulate nitrogen, and fecal pellet flux for trap deployed for December 2003-February 2004. * Range of values using a low and intermediate volume to carbon literature conversion.

SAMPLE	Date	TMF	BSi flux		OC flux		N flux		Bsi/OC	C/N	Fecal Pellets
		mg m^{-2} d^{-1}	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	w/w	w/w	mg C m^{-2} d^{-1} *
CALLINECTES 1	12/30/2003	13.8	0.2	1.7	5.0	36.0	0.7	4.9	0.0	7.3	0.65-2.0
CALLINECTES 2	1/1/2004	4.4	0.4	9.4	1.4	32.7	0.1	3.4	0.3	9.6	
CALLINECTES 3	1/3/2004	2.2	0.2	7.7	0.7	32.6	0.1	4.2	0.2	7.8	
CALLINECTES 4	1/5/2004	59.5	0.0	0.1	5.1	8.5	0.7	1.2	0.0	6.8	0.06 - 0.21
CALLINECTES 5	1/7/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	
CALLINECTES 6	1/9/2004	7.0	0.1	1.9	2.7	38.9	0.3	4.9	0.0	7.9	0.32-1.6
CALLINECTES 7	1/11/2004	25.5	0.2	0.6	3.2	12.4	0.4	1.6	0.0	7.6	
CALLINECTES 8	1/13/2004	5.0	0.1	1.4	2.1	42.5	0.3	5.8	0.0	7.3	
CALLINECTES 9	1/15/2004	3.6	0.1	3.3	1.8	50.6	0.3	7.1	0.1	7.1	
CALLINECTES 10	1/17/2004	19.6	0.2	0.9	1.9	9.5	0.3	1.5	0.1	6.2	
CALLINECTES 11	1/19/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	
CALLINECTES 12	1/21/2004	28.9	2.1	7.4	3.7	12.9	0.6	2.0	0.6	6.5	
CALLINECTES 13	1/23/2004	5.9	1.1	18.4	1.6	27.0	0.2	3.9	0.7	7.0	0.78-2.4
CALLINECTES 14	1/25/2004	9.1	2.1	22.9	1.8	20.1	0.3	2.8	1.1	7.1	
CALLINECTES 15	1/27/2004	3.9	0.8	19.7	0.6	15.6	0.1	1.9	1.3	8.3	
CALLINECTES 16	1/29/2004	7.7	1.6	20.7	1.3	17.0	0.2	2.4	1.2	7.1	
CALLINECTES 17	1/31/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	
CALLINECTES 18	2/2/2004	nd	nd	nd	nd	nd	nd	nd	nd	nd	
CALLINECTES 19	2/4/2004	5.6	0.5	9.3	1.2	21.5	0.2	2.9	0.4	7.5	0.13-0.42
Total Flux		376.0	18.9		58.4		8.1				

Table 10. Total mass flux, biogenic silica, organic carbon, particulate nitrogen, and fecal pellet flux for trap deployed for December 2004-February 2005. * Range includes a low and intermediate volume to carbon conversion from the literature.

SAMPLE	Date	TMF	BSi flux		OC flux		N flux		Bsi/OC	C/N	Fecal Pellets
		mg m^{-2} d^{-1}	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	w/w	w/w	mg C m^{-2} d^{-1*}
XIPHIAS 1	12/25/2004	2.1	0.2	8.5	1.3	62.1	0.2	10.2	0.1	6.1	0.002-0.008
XIPHIAS 2	12/27/2004	6.0	0.5	7.8	3.2	52.6	0.5	8.1	0.1	6.5	
XIPHIAS 3	12/30/2004	4.1	0.4	9.2	1.4	35.5	0.2	4.4	0.3	8.1	
XIPHIAS 4	1/1/2005	25.3	1.8	7.2	5.3	21.2	0.6	2.5	0.3	8.5	
XIPHIAS 5	1/3/2005	36.6	4.2	11.4	12.2	33.4	1.1	3.1	0.3	10.7	1.5-4.7
XIPHIAS 6	1/5/2005	24.2	2.7	11.0	5.6	23.3	0.7	2.8	0.5	8.2	
XIPHIAS 7	1/7/2005	4.8	0.5	11.2	1.1	23.8	0.2	3.2	0.5	7.5	
XIPHIAS 8	1/9/2005	3.4	0.5	14.1	1.1	32.1	0.2	4.6	0.4	7.0	0.01-0.31
XIPHIAS 9	1/12/2005	2.7	0.3	12.7	1.1	40.2	0.2	7.1	0.3	5.6	
XIPHIAS 10	1/14/2005	7.2	0.9	12.7	2.5	35.0	0.4	5.1	0.4	6.9	
XIPHIAS 11	1/16/2005	5.8	0.9	14.6	1.8	30.1	0.3	4.3	0.5	7.0	
XIPHIAS 12	1/18/2005	16.2	1.7	10.4	4.3	26.4	0.6	3.7	0.4	7.1	
XIPHIAS 13	1/20/2005	7.6	0.9	11.6	2.9	37.8	0.4	5.3	0.3	7.1	
XIPHIAS 14	1/22/2005	27.1	0.7	2.7	4.3	15.8	0.7	2.4	0.2	6.5	
XIPHIAS 15	1/25/2005	3.0	0.5	16.7	0.9	28.7	0.1	4.5	0.6	6.3	0.13-0.39
XIPHIAS 16	1/27/2005	5.8	0.7	12.7	1.6	27.4	0.2	4.2	0.5	6.5	
XIPHIAS 17	1/29/2005	5.6	0.7	12.8	2.6	46.8	0.5	8.5	0.3	5.5	0.20-0.64
Total Flux		375.1	36.2		106.5		14.0				

Table 10., ctd. Total mass flux, biogenic silica, organic carbon, particulate nitrogen, and fecal pellet flux for trap deployed for December 2004-February 2005. * Range includes a low and intermediate volume to carbon conversion from the literature.

SAMPLE	Date	TMF	BSi flux		OC flux		N flux		Bsi/OC	C/N	Fecal Pellets
		mg m^{-2} d^{-1}	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	mg m^{-2} d^{-1}	% TMF	w/w	w/w	mg C m^{-2} d^{-1}*
CALLINECTES 1	12/23/2004	2.2	0.1	6.6	1.8	84.1	0.2	9.3	0.1	9.0	
CALLINECTES 2	12/25/2004	3.0	0.1	3.6	1.1	36.3	0.2	5.7	0.1	6.4	0.04-0.11
CALLINECTES 3	12/27/2004	1.8	0.1	5.2	1.5	82.0	0.2	12.8	0.1	6.4	
CALLINECTES 4	12/30/2004	6.1	0.3	4.3	2.4	39.4	0.3	4.7	0.1	8.3	
CALLINECTES 5	1/1/2005	6.9	0.2	2.8	0.1	2.1	2.7	39.6	1.3	0.1	
CALLINECTES 6	1/3/2005	12.7	0.3	2.1	3.1	24.4	0.3	2.7	0.1	9.0	
CALLINECTES 7	1/5/2005	9.5	0.5	5.5	3.5	36.8	0.4	4.6	0.2	8.0	
CALLINECTES 8	1/7/2005	4.7	0.2	4.9	1.8	39.1	0.3	5.9	0.1	6.6	
CALLINECTES 9	1/9/2005	29.0	2.1	7.3	8.0	27.7	1.1	3.9	0.3	7.1	0.56-1.8
CALLINECTES 10	1/12/2005	2.8	0.5	17.1	2.2	79.1	0.3	11.3	0.2	7.0	
CALLINECTES 11	1/14/2005	23.4	2.6	11.0	6.0	25.7	0.9	3.7	0.4	7.0	
CALLINECTES 12	1/16/2005	13.2	1.7	13.1	4.5	34.4	0.7	5.2	0.4	6.6	
CALLINECTES 13	1/18/2005	23.8	3.6	15.3	5.9	25.0	0.9	3.7	0.6	6.8	
CALLINECTES 14	1/20/2005	58.3	4.7	8.1	8.3	14.2	1.2	2.0	0.6	7.1	1.4-4.3
CALLINECTES 15	1/22/2005	23.9	3.7	15.4	5.5	23.0	0.8	3.2	0.7	7.1	
CALLINECTES 16	1/25/2005	6.4	0.9	14.2	1.9	30.2	0.3	4.4	0.5	6.9	
CALLINECTES 17	1/27/2005	19.3	2.5	12.9	4.4	22.8	0.6	3.3	0.6	6.9	
CALLINECTES 18	1/29/2005	35.9	2.7	7.4	4.3	12.0	0.7	2.1	0.6	5.8	1.1-3.5
Total Flux		565.6	53.6		133.1		24.3				

Table 11. Regression results for total mass flux (TMF), biogenic silica (BSi), particulate carbon (POC), and particulate nitrogen (PN).

Parameter	R^2	p
Total Mass Flux vs. BSi	0.38	<0.0001
Total Mass Flux vs. POC	0.37	<0.0001
Total Mass Flux vs PN	0.13	0.003
BSi vs. POC	0.45	<0.0001
BSi vs. PN	0.17	0.0004

Figure 11. Mooring diagram of *Xiphias* deployment during December 2003. Courtesy of Dr. Vernon Asper.

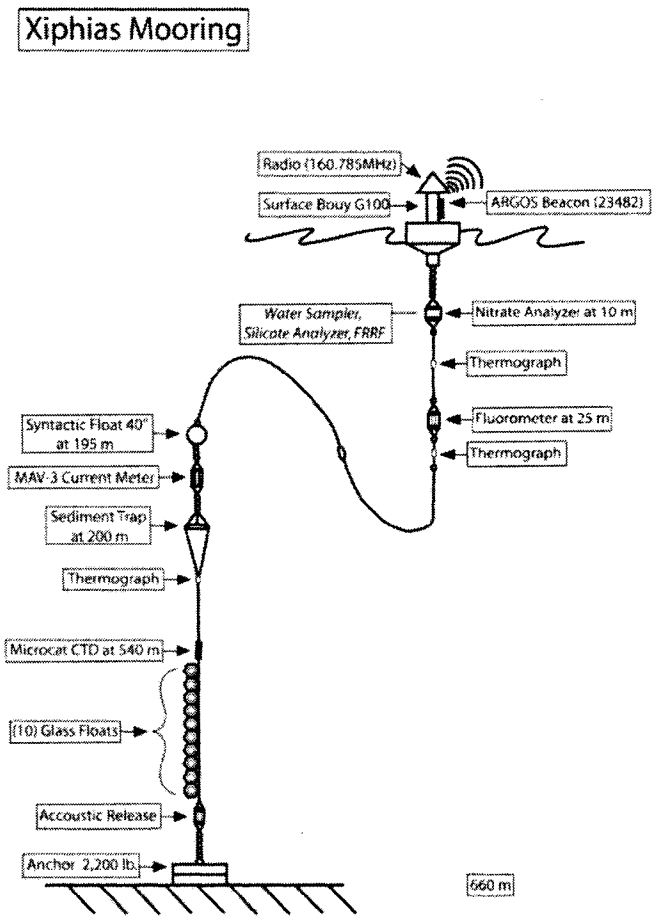


Figure 12. Total Mass Flux (TMF), Biogenic Silica (BSi), and Organic Carbon (OC) Flux for *Xiphias* mooring in a) December 2003-2004, b) December 2004-2005, and *Callinectes* mooring in c) December 2003-2004, and d) December 2004-2005.

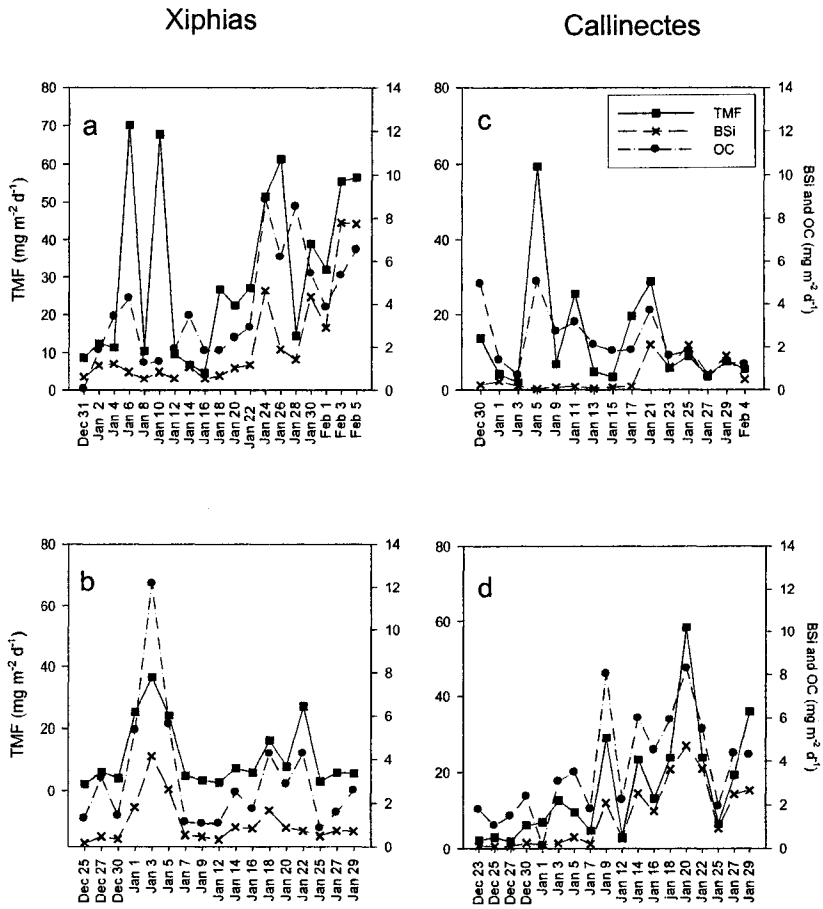


Figure 13. Particulate organic carbon and particulate nitrogen linear relationship for all data for IVARS 2003-2004 and 2004-2005.

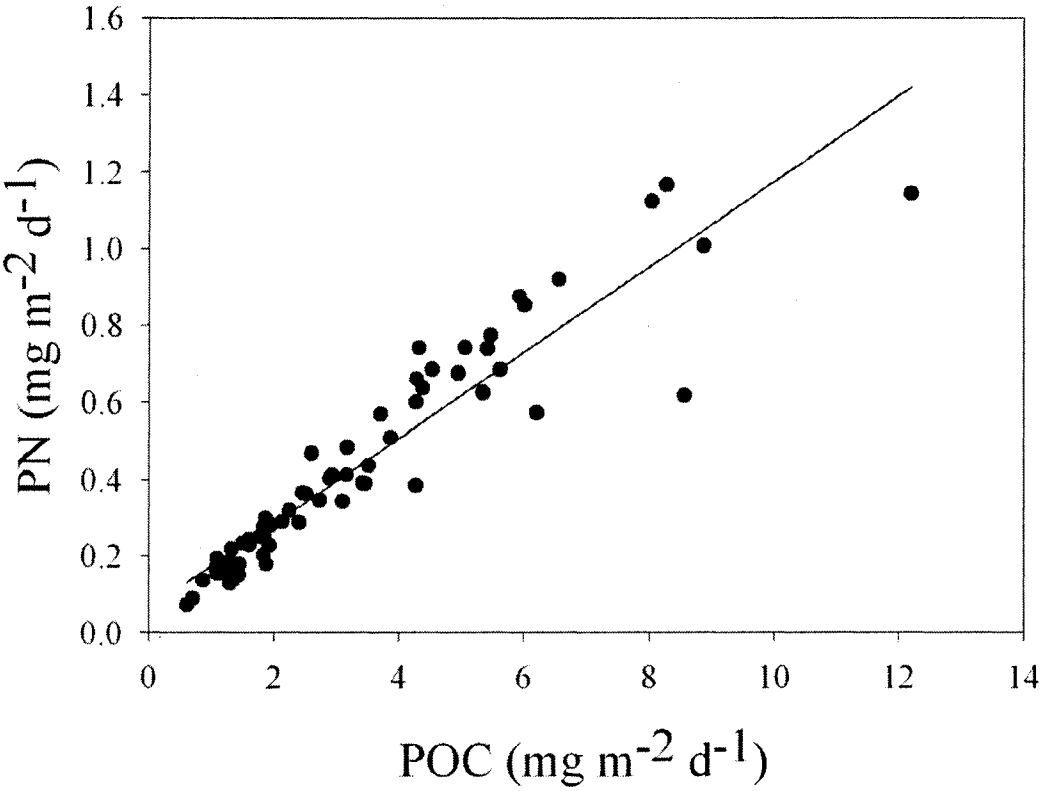


Figure 14: Fecal pellet flux contribution to total particulate organic carbon (POC) flux for the *Xiphias* mooring in a) December 2003-2004, b) December 2004-2005, and *Callinectes* mooring in c) December 2003-2004, and d) December 2004-2005. Open circles represent fecal pellet POC flux and close circles represent total POC flux.

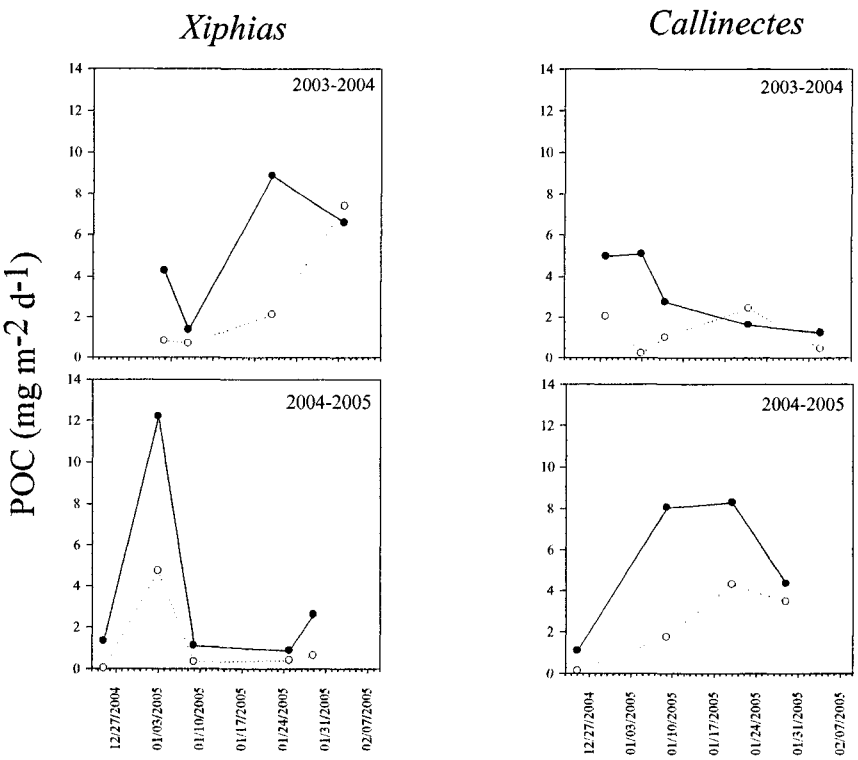
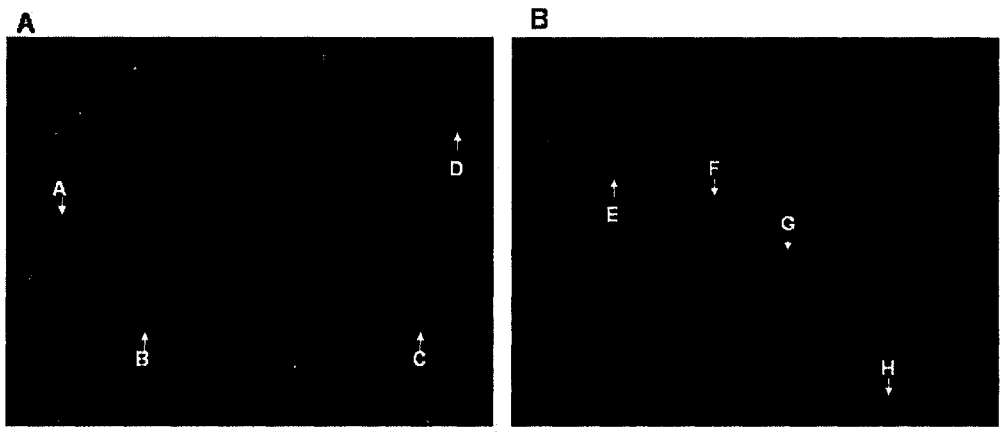


Figure 15a: Examples of shape and color for fecal pellet flux during IVARS. This sample was taken from *Callinectes* on December 2003. Four types of pellets can be seen with A) representing lighter brown pellets, B) dark brown pellets, C) white spherical objects that may be tests, and D) light brownish white pellets. b) Example of shape and color for fecal pellet from taken from *Xiphias* on January 10, 2004. Four types of pellets can be viewed with E) representing a bright green pellet, F) brown/green pellet, G) whitish green pellet, and H) aggregate. C) Image taken at 63X. c) Fecal pellets from *Callinectes* sample 18 during last sampling period on January 29, 2005. This station had over 100% of total sediment trap flux represented by fecal pellets.



Section IV. Photosynthesis/irradiance relationships of solitary and colonial forms of *Phaeocystis antarctica* from the Ross Sea

ABSTRACT

Understanding environmental controls on the composition of phytoplankton assemblages is critical because taxonomic structure is a major determinant of energy flow within food webs and biogeochemical cycles. In the Ross Sea there are two major phytoplankton functional groups: diatoms and prymnesiophytes, with the latter being dominated by *Phaeocystis antarctica*. It has been suggested that this relatively simple phytoplankton assemblage composition results from a differential photosynthetic response of the two groups to irradiance, with *P. antarctica* being able to more effectively photosynthesize (and presumably grow) at lower irradiances than diatoms but this has yet to be shown experimentally. A comparison of the growth characteristics of flagellate and non-flagellate forms of *Phaeocystis* sp. is crucial in understanding their large ecological impact on ecosystems and temporal and geographical distributions. There are few data from the Southern Ocean on the relative photosynthetic responses of colonies (non-flagellate) and solitary (flagellate and non-flagellate) forms of *P. antarctica*. The goals of this study are to assess the relative photosynthetic potential of solitary and colonial *P. antarctica* cells and mixed phytoplankton assemblages in December of 2001, 2003, and 2004 and to determine the magnitude of interannual variability of photosynthetic parameters in the Ross Sea. Interannual variations in maximum photosynthesis rates (P_m^B) were observed between December 2001 and December 2003. This variability was found for both the >20 (colonial) and >0.7 μm (total) size fractions. The total phytoplankton assemblage (>0.7 μm) and larger cells (> 20 μm) also had a higher maximum photosynthetic rate in December 2001 than December 2003 suggesting maximum photosynthetic rates were highest when solitary *P.*

antarctica cells had the highest abundance relative to colonial forms. Lab experiments showed that growth stage affects the maximum photosynthesis rates of colonial *P. antarctica.* During nutrient-replete conditions, colonial cells had higher maximum photosynthetic rates than solitary cells, which may be one reason for the high abundance of colonies during bloom formation.

INTRODUCTION

Ross Sea, Antarctica phytoplankton blooms

Understanding environmental and oceanographic controls on the composition of phytoplankton assemblages is critical because taxonomic structure is a major determinant of energy flow within food webs (Boyd and Newton, 1999; Archer et al., 2000). The Ross Sea polynya (an area of reduced ice cover surrounded by ice) has somewhat predictable phytoplankton blooms due to the physical properties of annual sea ice retreat and water stratification (Arrigo et al., 1999), with a seasonal chlorophyll maxima of over 10-15 $\mu g\ L^{-1}$ (Smith et al., 2000). Abiotic properties such as light, temperature, and dissolved nutrients control biomass, primary production and phytoplankton growth. The Ross Sea seasonal phytoplankton bloom is one of the largest blooms in the Southern Ocean, with spatial coverage of ca. 187,000 km^2 (Smith and Nelson, 1985; Comiso et al., 1993; Sullivan et al., 1993). Although interannual variability occurs in this region, seasonal effects appear to be greater than interannual production cycles (Smith et al., 2000).

In the Ross Sea there are two major functional phytoplankton groups: diatoms and prymnesiophytes, with the latter being dominated by *Phaeocystis antarctica*. It has been suggested that this relatively simple phytoplankton assemblage composition results from a differential photosynthetic response of the two groups to irradiance, with *P. antarctica* being able to more effectively photosynthesize (and presumably grow) at lower irradiances than diatoms (Arrigo et al., 1999). Arrigo et al. (1999) and Smith and Asper (2001) found that *P. antarctica* abundance covaried with deeper mixed layers (and hence lower irradiances). However, van Hilst and Smith (2002) were unable to statistically

distinguish the measured photosynthetic response of each group, and concluded that other factors were important in generating the spatial differentiation. They suggested that the micronutrient iron might play an important role as it is reduced to extremely low concentrations by biological removal during the austral summer.

Iron has been shown to seasonally limit phytoplankton growth within the Ross Sea. Sedwick and DiTullio (1997) and Sedwick et al. (2000) conducted iron enrichment experiments and found a dramatic response to iron additions, and concluded that iron can limit phytoplankton growth. They speculated that variations in the influence of ice melt (and input of iron from ice) might give rise to spatial variations in assemblage composition. Olson et al. (2000) found that all species tested using pump-during-probe fluorescence exhibited strong iron limitation, but also noted that the variability observed within *P. antarctica* was far greater than that seen in other species. Subsequent studies have speculated that iron plays a critical role in the distribution and growth of the extant functional groups as well (Smith et al., 2000; Smith and Asper, 2001; Arrigo et al., 2003; Smith et al., 2006).

Phaeocystis antarctica

The size structure of phytoplankton assemblages also drives marine pelagic food web dynamics (Legendre and Le Fèvre, 1991) and export (Tremblay and Legendre, 1994). Three species of *Phaeocystis*, including *P. antarctica*, exist as single, flagellated cells or as non- flagellated cells in colonies (Lancelot et al., 1998; Rousseau et al., 1994). It is known that there are several life stages in which a motile cell with flagella can form into hollow, spherical colonies with an effective spherical diameter greater than 1 mm and with active division of the cells within the matrix (Mathot et al., 2000). As colonies

sink through the water column, solitary cells can be released from the colonial matrix, leaving a large number of flagellated single cells (Wassman et al., 1990). Although little is known about what environmental factors control colony formation and release of solitary cells from colonies, inorganic nutrient concentrations have been speculated to influence the ratio of the form of *Phaeocystis* sp. (Verity et al., 1988).

Mathot et al. (2000) found that spatial and temporal trends in relative abundance occurred between solitary and colonial cells in the southern Ross Sea. Most *P. antarctica* cells were associated with colonies during the austral spring through the time of maximum biomass (mid-December), and thereafter the number of solitary cells began to increase. Smith et al. (2003) investigated the percentage of cells associated with colonies in 1996-7, and found that < 10% were colonial in late October, more than 98% of the cells were colonial at the time of the maximum chlorophyll concentration (mid-December), which, subsequently declined to ~50% in colonies in late February. Integrated over the entire growing season, flagellated cells contributed 33% of the total *P. antarctica* abundance. Smith et al. (2003) also suggested that the abundance of solitary cells might be controlled by removal by microzooplankton and heterotrophic flagellates, whereas the growth and abundance of colonial cells may be controlled by iron. Because solitary cells can be released from colonies, it was suggested that the ratio of solitary to colonial cells represented a dynamic balance between grazing and nutrient limitation. The physiological differences of these forms might also allow them to respond to varying environmental conditions.

Mathot et al. (2000) also found that cell size and carbon content differed between the solitary and colonial *P. antarctica* cells, with solitary cells smaller and having only

25% of the carbon as colonial cells. While no relationship between *P. antarctica* size and physiological state has been shown, bacteria generally show a positive relationship between size and growth (i.e., more rapidly growing cells are larger; Oliver et al., 2004). This relationship has been confirmed for colonial and solitary cells of *P. globosa* and *P. pouchetii* (Veldhuis et al., 2005). If the same is true for *P. antarctica*, then colonial cells might be expected to have more rapid rates of growth, photosynthesis, and nutrient uptake. However, no comparison of the photosynthetic potential of the two morphotypes is available. Lancelot and Mathot (1985) also found that the mucous envelope formed acts as a reserve for the cells and was reabsorbed during the dark period during the colonial stages of other species of *Phaeocystis* (*P. pouchetii*). Therefore, part of the carbon fixed by *P. pouchetii* colonies can be allocated to extracellular carbon production (Lancelot and Mathot, 1985). *Phaeocystis* can acclimate to abrupt irradiance changes by xanthophyll cycling (Moisan et al., 1998), and by increasing the amount of pigment per cell to adapt to low light levels. These characteristics allow making *Phaeocystis* to dominate polar regions due to "bottom-up" controls (Moisan and Mitchell, 1999).

Photosynthesis/Irradiance Models

Physiological characteristics of phytoplankton correspond to parameters derived from photosynthesis and irradiance models. These characteristics are affected by environmental parameters such as nutrient concentrations, irradiance, and phytoplankton community composition. The relationship between photosynthesis and irradiance can be modeled using several mathematical relationships (eg., Webb et al., 1974; Jassby and Platt, 1976; Platt et al., 1980). These resulting parameters are critical for estimating and modeling phytoplankton photosynthesis and production (Sathyendranath et al., 1999).

P_m^B or theoretical maximum rate of production is controlled by light independent (dark) carbon fixation reactions. When normalized to chlorophyll, P_m^B is the light saturated rate of photosynthesis that is affected by cellular chlorophyll concentrations and enzyme activity during carbon fixation. Normalizing α or the light limited slope to chlorophyll relates it to the relative concentrations of PSII reaction centers.

The goals of this study are to assess the relative photosynthetic potential of solitary and colonial *P. antarctica* cells and mixed phytoplankton assemblages in December of 2001, 2003, and 2004 and to determine the magnitude of interannual variability of photosynthetic parameters in the Ross Sea. Although there have been studies on how *Phaeocystis* sp. and other phytoplankton groups compare with respect to photosynthesis, it is still not known how single and colonial cells differ. Since α is affected by physiological differences, a comparison between solitary and colonial forms will provide information on which form would perform better under lower light conditions. For example, picoplankton generally have larger α values so they perform better than larger phytoplankton deeper in the water column (eg., Pierson et al., 1992). Since P_m^B is more affected by temperature and nutrients (or dark reactions of photosynthesis), a difference could mean that the cell type is not at its optimum temperature or nutrient concentration.

METHODS

Study site and field measurements

Water samples were collected from the southern Ross Sea as part of the Interannual Variations in the Ross Sea (IVARS) program (Smith et al., 2006) that was

conducted from 2001-2005. Two cruises per year were completed (generally in December and February, representing the period of maximum biomass and the end of the growing season, respectively). Locations of stations where photosynthesis/irradiance (P/E) measurements were conducted are plotted in Figure 16. The euphotic zone was sampled using a SeaBird 911+ CTD/rosette system from which samples for nutrients and biomass estimates (chlorophyll *a* concentrations of the total assemblage, of those forms >0.7 μm , > 20 μm, and those < 20 μm; HPLC pigments, particulate organic carbon/nitrogen concentrations, biogenic silica concentrations, and samples for phytoplankton) were taken. For further discussion of data see Smith et al. 2006, section I, or http://www.vims.edu/bio/ivars). The depth of the euphotic zone (1% of surface irradiance) was determined from a BioSpherical Instruments PAR sensor mounted on the rosette. Samples for P/E experiments were collected from the 50% light depth.

Chlorophyll concentrations were determined by fluorescence after filtering the samples through Whatman GF/F filters and extracting in 90% acetone for 24 h at -20° C (Smith et al., 2006). Samples were read on a Turner Designs Model 10AU fluorometer that had been calibrated with a known concentration of commercially purified chlorophyll *a* (Sigma Chemical). Independent samples were size-fractionated by filtering through 20 μm polycarbonate filters (Poretics). For *P. antarctica*, the material retained on the filter was assumed to represent colonial cells, as the mean size of colonies is substantially greater than 20 μm (Mathot et al., 2000). The fraction that passed through the filters was assumed to represent solitary cells, although it also would contain small colonies. Phytoplankton pigments were determined by filtering a known volume through a GF/F filter, quick freezing the filter in liquid nitrogen (-80°C), and returning the

samples to the laboratory for analysis using high performance liquid chromatography (HPLC) on a Waters Millennium system. Full details of the HPLC procedure and results are provided in Smith et al. (2006).

Photosynthesis/Irradiance Method

Photosynthesis/irradiance relationships of solitary and colonial forms were determined by using a large-volume irradiance gradient incubator (Platt and Jassby, 1976). Twenty-three samples (265 mL each) were collected from one depth (generally that of the 50% isolume), to which ca. 100 μCi $NaH^{14}CO_3$ were added to each. The samples were added to the incubator, and the light (a high intensity xenon-arc light) was turned on (Figure 17). Surface seawater surrounded the samples and circulated through the incubator to maintain samples at ambient temperatures. Water also passed through a heat sink (5 cm thick) made of two plates of tempered glass. Because the light was mounted on one end of the incubator, a gradient of irradiance naturally occurred as distance from the light increased. A dark bottle (one bottle wrapped in aluminum foil) was used as a control. Irradiance was measured within each bottle while in the incubator with a BioSpherical Sensor quantum meter. Incubations lasted approximately 2 h. After incubation each sample was size-fractionated by filtering an aliquot of known volume through a 20 μm Poretics filter, and a separate volume though a GF/F filter. Only particulate organic carbon production was measured, and the exudation of DOM from phytoplankton was ignored. DOC release in two hours is generally less than 10% (Smith, personal communication). Therefore, a P/ E experiment that uses small whole water samples, will lead to higher estimates of productivity than those that involve the filtration of samples because DOC is included (Sakshaug et al., 1997). Each filter was placed in a

7 mL scintillation vial, and 0.25 mL of 10% HCL solution was added to remove any inorganic carbon on the filter. After ventilation for 24 h, 5 mL Ecolume® (ICN) was added, and after another 24 h in the dark, all samples counted on a liquid scintillation counter. Total available inorganic ^{14}C-bicarbonate was assessed by counting a 0.1 mL aliquot (to which 0.05 mL β-phenethylamine, a CO_2 trap, was added) directly in Ecolume.

Laboratory Culture experiments

In order to compare monocultures of *Phaeocystis antarctica* with field samples, *P. antarctica* (CCMP 1374) cultures were maintained at Crary Laboratory, McMurdo Station, Antarctica for 16 d in 2005-2006. A 50 L polycarbonate carboy with filtered (<0.2 μm) McMurdo Sound seawater was inoculated with 2 μg L^{-1} *P. antarctica* culture. The carboy was maintained at -2 °C with constant irradiance of 50 μmol m^{-2} s^{-1} to simulate natural conditions. Every four days, P/E experiments were performed in the same manner as the field samples with the incubation occurring in a -2° C environmental room to keep samples at a constant temperature. The photosynthetron used in the laboratory experiments utilized high output fluorescent lights rather than a xenon-arc light to minimize heat from the lamps on the samples. Photosynthesis-Irradiance models and analyses were performed as described for the field samples.

Data Analysis

Since photoinhibition was not significantly different from zero in our study, a widely used model without this term was used (modified Platt et al., 1980; Webb et al., 1974). Photosynthetic rates were fit to the Webb et al. (1974) empirical model:

$$P^B = P_s^B\left[1 - e^{-\alpha E / P_s^B}\right] \qquad \text{(Equation 1)}$$

where P^B = the rate of photosynthesis normalized to chlorophyll *a* [mg C (mg chl *a*)$^{-1}$ h^{-1}], P_s^B = the maximum rate of photosynthesis in the absence of photoinhibition, α = the initial, light-limited, linear photosynthetic rate [mg C (mg chl *a*)$^{-1}$ h^{-1} (μmol photons m^{-2} s^{-1})], and E = irradiance (μmol photons m^{-2} s^{-1}), The data were fit to this equation using SigmaPlot (Version 6). A derived parameter from these variables is E_k (the irradiance at which photosynthesis is saturated). It is derived by:

$$E_k = P_s^B / \alpha \qquad \text{(Equation 2)}$$

Phaeocystis antarctica dominated stations were identified based on the ratio of the accessory pigments fucoxanthin (for diatoms) and 19'-hexanoyloxyfucoxanthin. We believe this to be the best criterion because the amount of chlorophyll *a* per cell is far less for *P. antarctica* than for diatoms, so that diatoms contribute to biomass to a larger degree than their abundance would suggest. Regardless, cell abundances and ChemTax (which, derives taxonomic structure of phytoplankton from pigment ratios) also confirmed the large contribution of the prymnesiophyte relative to diatoms and for solitary and colonial cell abundances (Table 12; Smith et al. 2006; www.vims.edu/bio/ivars, Peloquin, unpublished).

It has been suggested that these accessory pigments cannot be used to quantitatively separate the diatoms and *P. antarctica*, as under cultured conditions the ratio of each pigment to chlorophyll, as well as the ratio to each other, was influenced by both irradiance and iron concentrations (van Leeuwe and Stefels, 1998). However, while

changes in the ratios of each to chlorophyll *a* have been observed in the Ross Sea as part of the temporal cycle of *P. antarctica* growth, little evidence exists for the conversion of fucoxanthin to 19'-hexanoyloxyfucoxanthin *in situ* (DiTullio and Smith, 1996; Smith and Asper, 2001; DiTullio et al., 2003). In our study we used these pigment ratios to select stations that were sampled within a very short time period (ca. four days). While there clearly could be some spatial variation in pigments and photophysiological adaptive state, the variations within each functional group are likely far smaller than observed in culture or over the entire growing season.

The parameter values resulting from the non-linear regressions (Webb et al., 1974) were compared using the general 2-way linear model of analysis of variance (ANOVA) after log transformation. A critical p value of 0.05 was selected a priori to evaluate the effects of temporal variation in the parameters and significant differences between size fractions. For the lab data, a comparison of the 95% Confidence Interval of the slopes was used to compare parameter values between size fractions.

RESULTS

Species Composition during field study

Microscopic and pigment data show that *P. antarctica* was most abundant during December of 2001 and 2003 (Table 12; Smith et al., 2006). The size structure of the *P. antarctica* assemblages differed between years. During December 2001, solitary, flagellated cells were the most abundant, whereas in 2003 colonial non-flagellated cells were most dominate in the assemblage (Peloquin, unpublished).

Interannual Variability in Size-Fractionated Photosynthesis

There were significant differences in size-fractionated photosynthesis for the >20 and >0.7 μm size fractions in this study (Figure 18). During December 2001 >20 μm P_m^B averaged 1.8 ± 0.7 mg C (mg chl a)$^{-1}$ h^{-1} for the four stations examined. Values ranged from 1.1-2.8 mg C (mg chl a)$^{-1}$ h^{-1} for the >20 μm size fraction (Table 13). The total phytoplankton assemblage (>0.7 μm) averaged 1.95 ± 0.33 mg C (mg chl a)$^{-1}$ h^{-1} and ranged from 1.75-2.45 mg C (mg chl a)$^{-1}$ h^{-1}. The solitary flagellates and phytoplankton <20 μm averaged 2.57 ± 0.77 mg C (mg chl a)$^{-1}$ h^{-1} and ranged from 2.08-3.46 mg C (mg chl a)$^{-1}$ h^{-1}. During December 2003, P_m^B averaged 0.51 ± 0.91 and ranged from 0.27-0.73 for the > 20 μm; 0.75 and ranged from 0.46-1.05 for the 0.7 μm; and 2.34 and ranged from 0.53-4.35 <20 μm size fraction. Finally, in December 2004, P_m^B averaged 1.11, 1.25, and 2.89 for the >20, 0.7, and <20 μm size fractions, respectively. P_m^B for December 2001 was significantly higher than December 2003 for the >20 and >0.7 μm size fractions (Table 13, 2 Way ANOVA, DF=50, F=5.42, p=0.0001). The bulk phytoplankton assemblage (>0.7 μm) and larger cells (> 20 μm) had a higher maximum photosynthetic rate in December 2001 than December 2003 (Figure 18). The small size fraction or solitary flagellates did not have significant interannual variation.

When comparing within size fractions each year, December 2001 size fractionated results did not show significant differences while in December 2003, the >20 μm size fraction P_m^B was significantly lower than the <20 μm size fraction (Figure 19). The December 2004 <20 μm assemblage also had significantly higher P_m^B than the bulk

and >20 μm size fractions. There were no significant interactions between year and size fraction (Figure 19, 2 Way ANOVA after log transformation, p=0.58).

The bulk initial light-limited rate of photosynthesis or α was also significantly higher in December 2001 than during the other two years (Table 13; Figure 20, 2-way ANOVA, after log transformation, F=5.15, p=0.0002). Also, in 2004, the <20 μm size fraction had a significantly higher α than the other size fractions (Figure 21). E_k (index of photoadaptation) was not significantly different interannually (Table 13, 2 Way ANOVA after log transformation, p>0.05). In order to determine if there was a significant relationship between P_m^B and α, Model II regressions were performed. The only size fraction to have a significant relationship was the >20 μm P_m^B vs α (Figure 22, R^2=0.972 p<0.0001).

P. antarctica colonies and maximum photosynthesis

A Model II Linear Regression was also performed in order to compare the relationship between percentage of colonial abundance and maximum photosynthesis (P_m^B) during December 2001 and 2003 when *Phaeocystis antarctica* was dominant (Figure 23, R^2=0.699 p=0.0007). Microscopic counts (Peloquin et al., in prep) and P_m^B values (this study) were combined with Joint Global Ocean Flux Study (JGOFS) measurements from 1996. Only stations that were dominated by *P. antarctica* (> 80%; using cell counts, and ChemTax delineation when available) and had P/E relationships (Hiscock, unpublished) and microscope counts (S. Mathot unpublished) were used in the regression. A total of five stations from the JGOFS stations were used from December

1996 and January 1997 along with eight stations from IVARS December 2001 and December 2003. A significant negative relationship was found between % *P. antarctica* colonial cells and P_m^B suggesting that when colonies are the most abundant that maximum photosynthesis is lowest in December and January.

McMurdo Time course

In order to assess whether there was a difference between size fractions for each time point in the McMurdo *P. antarctica* experiment, the 95% Confidence Intervals of the slopes/parameters of the P/E curves were compared. During the first eight days, the P_m^B was larger for the >20 μm size fraction (Figure 24). On the 12th day, the smaller size fraction was the same as the larger size fraction, and after the 16th day, the smaller size fraction P_m^B was larger than the >20 μm size fraction.

DISCUSSION

Implications of differences in interannual and size fractionated photosynthesis

A comparison of the growth characteristics of flagellate and non-flagellate forms of *Phaeocystis* sp. is crucial in understanding their large ecological impact on ecosystems and their temporal and geographical distributions (Lancelot and Rousseau, 1994; Peperzak et al. 2000). There are few data from the Southern Ocean on the relative responses of colonies (non-flagellate) and solitary (flagellate and non-flagellate) forms of *P. antarctica*. When we originally started this work, we expected to see substantial prymnesiophyte (*P. antarctica*) accumulations, as had been observed in the 1990's (Smith and Gordon, 1997; Arrigo et al., 1999; Smith et al., 2000; Smith and Asper, 2001). We were surprised by the mixtures of *P. antarctica* and diatoms, as well as the

substantial contribution of diatoms throughout the study during December 2004 and during February of our study (Section I; Smith et al., 2006). Thus, the number of stations where *P. antarctica* dominated the biomass (and both size fractions) was limited based on the criterion of van Hilst and Smith (2002) and as verified with microscope observations. Smith et al. (2006) found that in both 2001 and 2003 prymnesiophytes represented ca. 80% of the chlorophyll (ca. 6 $\mu g\ L^{-1}$) in December, and that the chlorophyll contributions of *P. antarctica* decreased markedly by February (to < 1 $\mu g\ L^{-1}$). Such rapid declines could be due to enhanced (and rapid) aggregate formation and passive sinking due to micronutrient limitation during the strongly stratified summer (Olson et al., 2000). If such declines were indeed initiated by the onset of iron limitation, it is logical that colonies would reflect that first, as single cells (by virtue of their surface area: volume relationship) would likely be less stressed under conditions of nutrient limitation than larger colonies (Smith et al., 2003). Iron limitation has also been shown to decrease P_m^B values (Lindley et al., 1995). The low P_m^B exhibited by colonial *P. antarctica* during December 2003 suggests they were under iron stress, but no iron measurements were taken during this study. Peloquin and Smith (2006) observed low maximum photochemical quantum yields (F_v/F_m) of 0.3-0.4 during the 2003 field season and suggested the phytoplankton blooms were under severe stress. The α for the bulk assemblage was also significantly higher in 2001 (Figure 21).

It has been observed that manganese, phosphate, and possibly iron (Davidson and Marchant, 1987; Lubbers et al., 1990; Veldhuis et al. 1991) can be sequestered in the colonial matrix and subsequently reused during later growth, thereby giving colonial a

competitive advantage over single cells during micronutrient limitation. It may be possible that other negative effects associated with colonies, rather than micronutrient limitation, are occurring in 2003. Robinson et al. (2003) argued that colonial bloom development could cease due to excessive colonial carbon requirements restricting colony size. These reductions in both forms would be consistent with the onset of iron limitation earlier in 2003 than 2001 (Smith et al., 2006). The results from this study also demonstrate the role of morphology in the bulk maximum photosynthetic rate when *P. antarctica* is dominant. As mentioned previously, studies have shown that *Phaeocystis* sp. colonies may be capable of higher growth rates than smaller solitary flagellates. But, studies of *P. antarctica* in the field are limited. Large phytoplankton are capable of higher growth rates or maximum carbon specific photosynthesis than small-sized phytoplankton, but studies are limited (Furnas, 1991; Frenette et al., 1996; Crosbie and Furnas, 2001; Cermeño et al., 2005). More rapid larger cell growth tends to be restricted to high-nutrient environments. During December, 2001 *P. antarctica* colonies were capable of the same rate of maximum photosynthesis as solitary cells. Like the previous studies, these results suggest that physiological factors could also explain the dominance of the colonial form of *P. antarctica* during December. While this contrasts with theoretical models suggesting that solute exchange increases with decreased cell radius, other physiological mechanisms such as nutrient storage or increasing scalable components may give the larger colonial *P. antarctica* cells an advantage (Raven, 1998).

Phytoplankton growth stage

Colonial cells of *P. globosa* have been found to divide at the same rate as motile cells. However, it has been shown that while other species, such as *P. pouchetti,* have

lower colonial specific growth rates reflecting that their mucilaginous matrix lacking as an energy reservoir (Baumann et al., 1994; Lancelot et al., 1991). SeaWiFS plots of chlorophyll show that in December 2001, we sampled during the early bloom stage while in December 2003, the IVARS sampling was about two weeks later. So, we sampled the bloom after the maximum biomass was reached (Peloquin, unpublished; Figure 25).

Effects of growth stage on maximum photosynthesis

The experiments with the *P. antarctica* monoculture also help illustrate how the magnitude of P_m^B compares temporally between size fractions (Figure 24). At the end of the 16-d experiment, P_m^B for the <20 μm size fraction was higher than for the larger size fractions. In contrast, P_m^B was much higher for the colonial size fraction than the solitary cells at the beginning of the experiment. Nutrients (nitrate) were limited or below detection in some cases after the 16 d period, suggesting that nutrient limitation may be allowing solitary *P. antarctica* cells to compete better as long as sufficient light is available.

Relationship between maximum photosynthetic rate and α

The variations in E_k observed in our study could indicate that each morphotype is capable of maintaining a balance between the light and dark reactions of photosynthesis. The bulk and solitary flagellates did not have significant relationship between α and P_m^B; this may reflect photoacclimation, and the assemblages could be experiencing "E_k dependent variability" which, involves independent changes of the light-limited slope (α) and P_m^B (Behrenfeld et al., 2004). It is generally assumed that α (when

normalized to chlorophyll concentration) is relatively constant (Steemann Nielsen and Jørgensen, 1968). In our study there were differences in α between years (with December 2001 > 0.7 μm size fractions being significantly higher than other years), and size fractionated photosynthesis significantly different in December 2004 than other years (Figure 19, 20). This could be due to the cells changing concentrations of photosynthetically active accessory pigments, photoinhibition, photosystem I: photosystem II ratio, or nonphotochemical quenching (Behrenfeld et al., 2004). Future experiments will be need to examine the mechanisms by which *P. antarctica* α varies.

Conclusions

The forms of *P. antarctica* exist in a dynamic equilibrium in nature, and a distinct temporal trend occurs in these forms. Different controls of each form exist, and hence the relative importance of these controls (bottom-up controls on colonies vs. top-down controls on small flagellates) as well as any differential growth between them will ultimately regulate their biomass within a bloom. Our data are relatively limited, but also represent some of the first field data showing differences in the photosynthetic parameters between the morphotypes of *P. antarctica*. Further study will clarify the importance of these differences, as well as the environmental and ecological regulation of the exchanges between the two. Understanding these differences will allow greater insights into the influence of the biotic composition on biogeochemical cycles in the Ross Sea.

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Table 12. The concentration of chlorophyll, its distribution among size classes, and the concentrations of accessory pigments at the stations where photosynthesis/irradiance measurements were completed during December. Where there are no pigment data available from the IVARS database (http://www.vims.edu/bio/ivars); stations selected based on microscopy (Peloquin, unpublished) and were considered to be dominated by *Phaeocystis antarctica* when abundance was >80%. A few stations during December 2004 did not fit into either category and were included in the regression analyses. Data that was not collected is marked as nd.

Year	>0.7µm Chlorophyll (µg L^{-1})	>20µm Chlorophyll (%)	19-Hex (µg L^{-1})	Fuco (µg L^{-1})	Ratio (Hex/Fuco)	Prymnesiophyte ChemTax (%)	Microscopic Observations *Phaeocystis antarctica* %
2001 Stations							
1	2.18	101.8	0.63	0.32	1.96	83.0	98.9
2	4.75	64.6	3.87	0.82	4.71	79.4	96.0
7	8.52	18.0%	10.28	1.51	6.80	87.5	95.9
10	5.56	39.6	7.44	1.33	5.58	81.9	90.4
2003 Stations							
2	10.35	57.7	nd	nd	nd	nd	nd
5	4.11	74.9	5.68	1.61	3.52	85.7	94.5
9	3.15	90.2	2.42	0.86	2.82	85.7	85.9
12	1.99	81.4	nd	nd	nd	nd	80.9
2004 Stations							
1	6.82	82.0	nd	nd	nd	nd	nd
2	2.81	45.9	1.04	5.88	0.18	62.4	nd
3	10.92	80.5	0.53	11.66	0.05	33.1	0.3
4	1.16	22.5	0.12	1.07	0.11	35.9	nd
6	11.1	84.9	0.26	9.91	0.03	19.6	0.3
10	9.83	85.3	0.16	3.85	0.04	nd	nd
13	8.96	79.4	0.06	3.44	0.02	nd	0
17	4.13	84.8	nd	nd	nd	nd	nd
19	4.04	35.2	0.50	3.28	0.15	nd	nd
21	0.74	49.3	0.19	0.94	0.20	nd	nd

Table 13. Maximum photosynthetic rates, light limited rates, and light-saturation index for the three size fractions (> 20, >0.7 and < 20 µm, corresponding to colonial and solitary forms of *P. antarctica*) stations dominated by *Phaeocystis antarctica* in December 2001, 2003 and 2004. Values generated from Photosynthesis/Irradiance model and error represents standard error of each parameter. Parameters that were not significant ($p>0.05$) are labeled with ns. Units of P^b_{max}: mg C (mg chl)$^{-1}$ h^{-1}; α: mg C (mg chl)$^{-1}$ h^{-1} (µmol photons m^{-2} s^{-1})$^{-1}$: E_k: µmol photons m^{-2} s^{-1}.

Year	>20µm P^b_{max}	>0.7µm P^b_{max}	<20µm P^b_{max}	>20µm α	>0.7µm α	<20µm α	>20µm E_k	>0.7µm E_k	<20µm E_k
2001									
1	1.5 ± 0.2	1.8 ± 0.5	ns	0.03 ± 0.006	ns	ns	46	ns	ns
2	1.1 ± 0.1	1.8 ± 0.2	3.5 ± 0.7	0.03 ± 0.009	ns	ns	38.9	ns	ns
7	2.8 ± 0.3	2.5 ± 0.4	2.2 ± 0.6	0.06 ± 0.02	0.04 ± 0.02	ns	49.7	58.5	ns
10	1.7 ± 0.2	1.8 ± 0.1	2.1 ± 0.2	0.03 ± 0.01	0.15 ± 0.05	0.3± 0.1	51.4	12.6	7.4
Average	*1.8 ± 0.7*	*1.9 ± 0.3*	*2.6 ± 0.8*	*0.04 ± 0.01*	*0.09 ± 0.07*	*0.3*	*47.2 ± 5.6*	*35.6 ± 32.4*	*7.4*
2003									
2	0.6 ±0.04	0.5 ±0.03	0.5 ± 0.1	0.007 ± 0.001	0.008 ± 0.001	0.007 ± 0.002	73.7	57.8	76.3
5	0.5 ± 0.03	0.9 ± 0.01	2.0 ± 0.2	0.009 ± 0.001	0.01 ± 0.001	0.02 ± 0.005	52.1	62.4	88.5
9	0.3 ± 0.03	0.6 ± 0.05	4.4 ± 0.5	0.006 ± 0.001	0.02 ± 0.003	0.1 ± 0.03	42.3	39.9	43.4
12	0.7 ± 0.03	1.1 ± 0.1	2.4 ± 0.4	0.02 ± 0.002	0.02 ± 0.005	0.06 ± 0.03	46.8	47.1	40.5
Average	*0.5 ± 0.2*	*0.8 ± 0.3*	*2.3 ± 1.6*	*0.01 ± 0.004*	*0.02 ± 0.006*	*0.05 ± 0.04*	*53.7 ± 10.2*	*51.8 ± 10.2*	*62.2 ± 23.9*
2004									
1	0.6 ± 0.08	1.4 ± 0.1	5.1 ± 0.7	0.007 ± 0.001	0.02 ± 0.002	0.06 ± 0.01	87.6	81.2	79.3
2	0.6 ± 0.05	3.1 ± 11.6	5.2 ± 1.2	0.005 ± 0.001	0.02 ± 0.004	0.03 ± 0.006	116.9	172.3	182.3
3	0.8 ± 0.05	1.1 ± 0.1	2.6 ± 0.2	0.009 ± 0.001	0.02 ± 0.001	0.05 ± 0.007	89.1	68.6	55.8
4	1.4 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	0.04 ± 0.01	0.04 ± 0.02	ns	37.1	31.7	ns
6	ns	0.7 ± 0.1	1.0 ± 0.3	ns	0.02 ± 0.001	ns	ns	40.15	ns
10	2.6 ± 0.2	0.7 ± 0.04	2.6 ± 0.2	0.1 ± 0.02	0.02 ± 0.002	0.1 ± 0.02	24.2	27.2	24.2
13	ns	1.0 ± 0.1	4.2 ± 0.7	0.003 ± 0.001	0.03 ± 0.007	0.1 ± 0.05	ns	35.7	36.6
17	0.3 ± 0.03	0.6 ± 0.03	1.9 ± 0.1	0.004 ± 0.001	0.01 ± 0.001	0.05 ± 0.006	68.7	46.6	40.5
19	0.5 ± 0.03	1.0 ± 0.1	3.9 ± 0.4	0.008 ± 0.001	0.02 ± 0.003	0.09 ± 0.02	62.3	47.0	42.1
21	2.1 ± 0.2	1.6 ± 0.11	1.3 ± 0.2	0.04 ±0.005	0.03 ± 0.003	0.02 ± 0.004	54.1	63.2	79.5
Average	*1.1 ±0.8*	*1.3 ± 0.7*	*2.9 ± 1.6*	*0.02 ± 0.03*	*0.02 ± 0.008*	*0.06 ± 0.03*	*67.5 ± 30.1*	*61.4 ± 42.6*	*67.5 ± 50.5*

Figure 16. Location of the stations sampled for *Phaeocystis antarctica* photosynthesis/irradiance experiments.

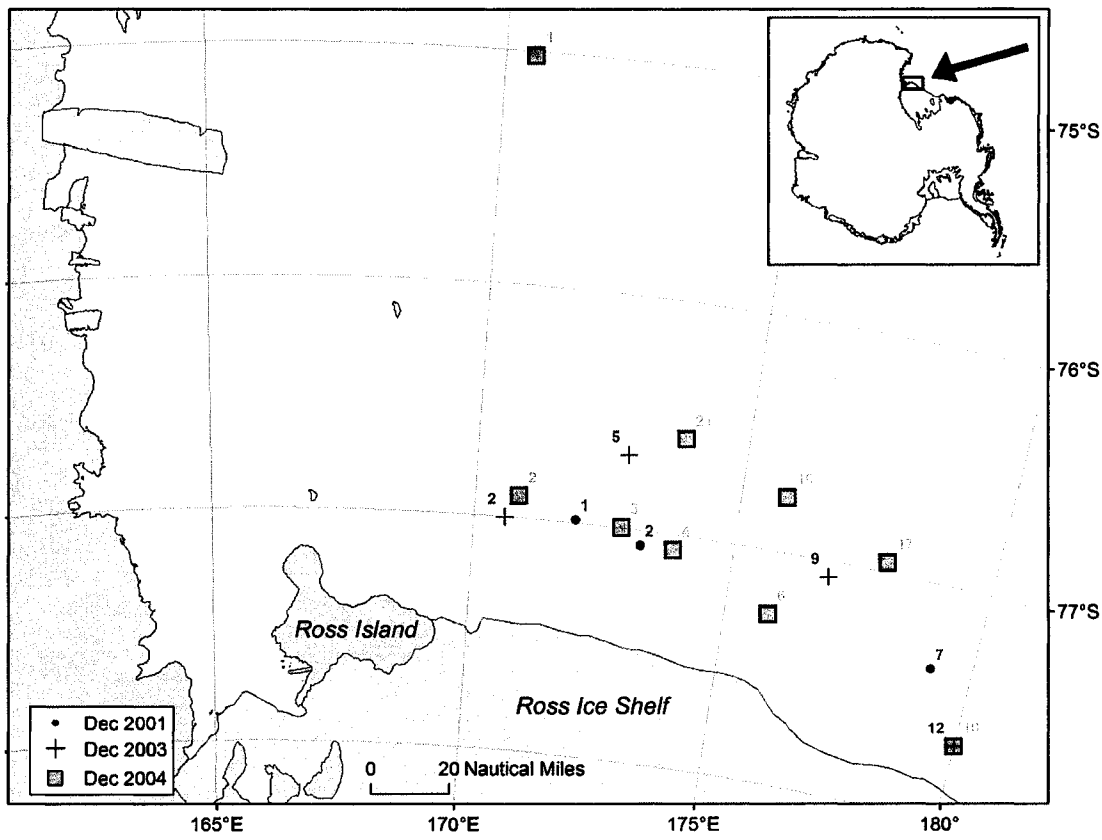


Figure 17. Photosynthetron that was utilized in IVARS experiments with Quartz-Xenon lamp which was located on the right side of the holding tank for the Qorpaks. This provided a gradient of irradiance (brighter to darker). The holding tank was circulated continuously with *in situ* seawater to keep samples at a constant temperature. Drawing is not to scale.

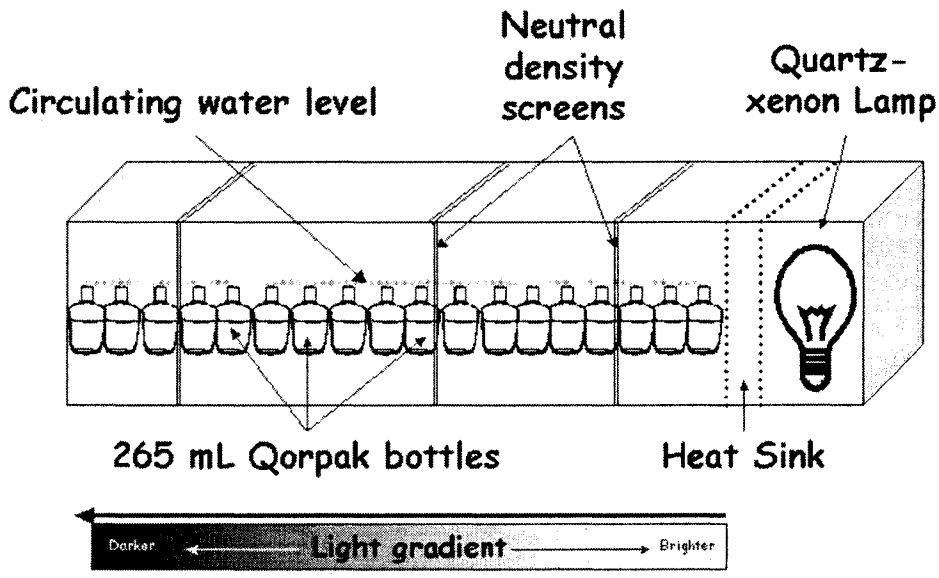


Figure 18. Interannual variation in size fractionated photosynthesis (>20, >0.7, and <20 μm) for IVARS transects during December 2001, 2003 and 2004. Letters denote significant differences.

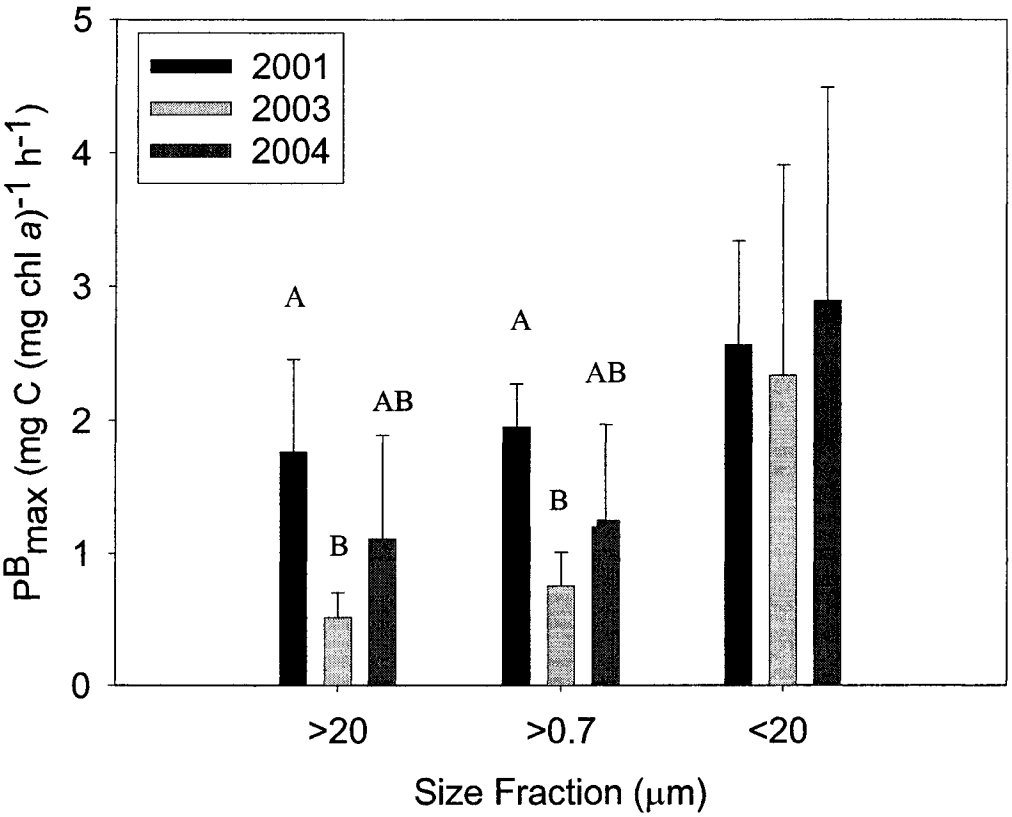


Figure 19. Variation in size fractionated photosynthesis (>20, >0.7, and <20 μm) for IVARS transects December 2001, 2003 and 2004. Letters denote significant difference.

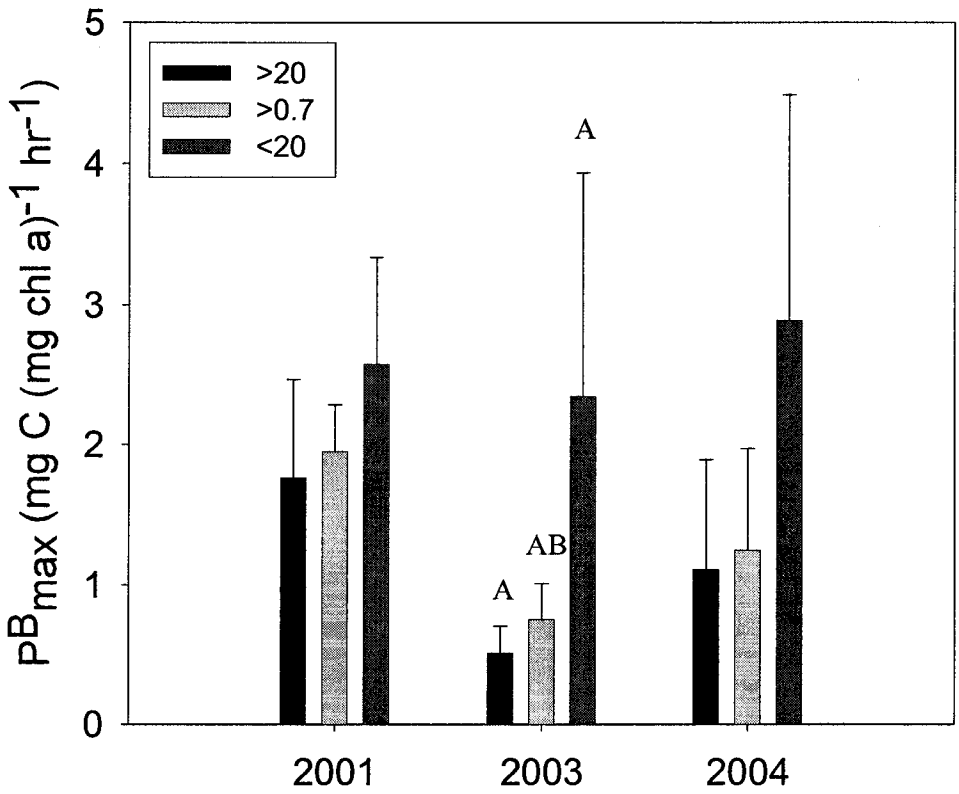


Figure 20. Interannual variation in α (light limited slope) (>20, >0.7, and <20 μm) for IVARS transects December 2001, 2003 and 2004. * denotes a significant difference between the treatments.

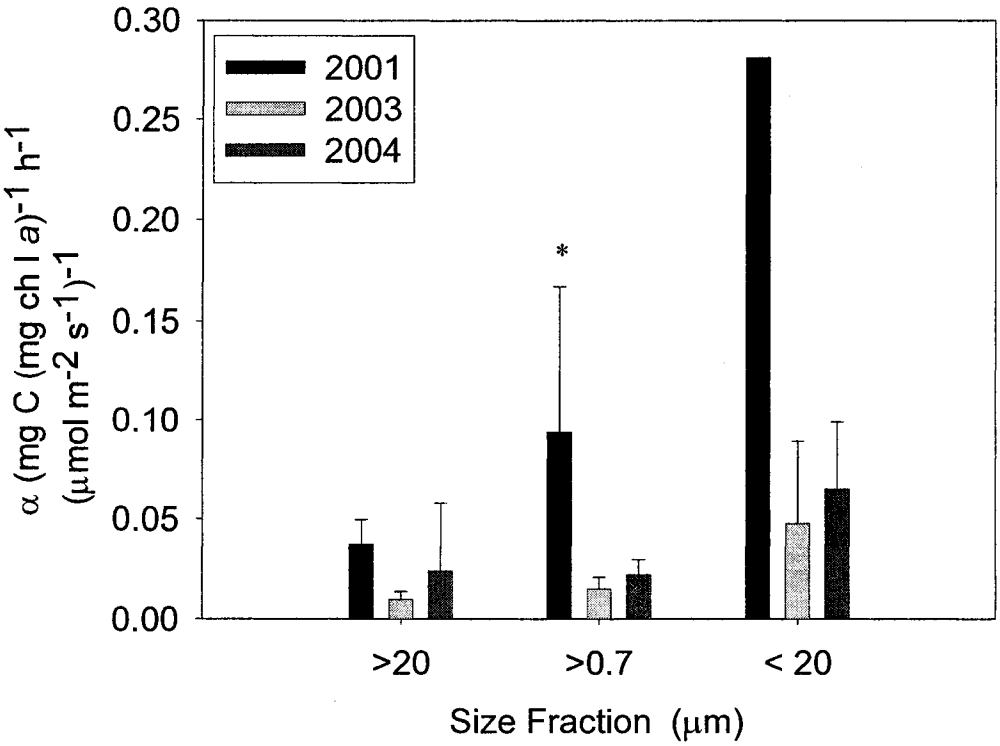


Figure 21. Variation in α (>20, >0.7, and <20 μm) for IVARS transects December 2001, 2003 and 2004. * denotes a significant difference between treatments.

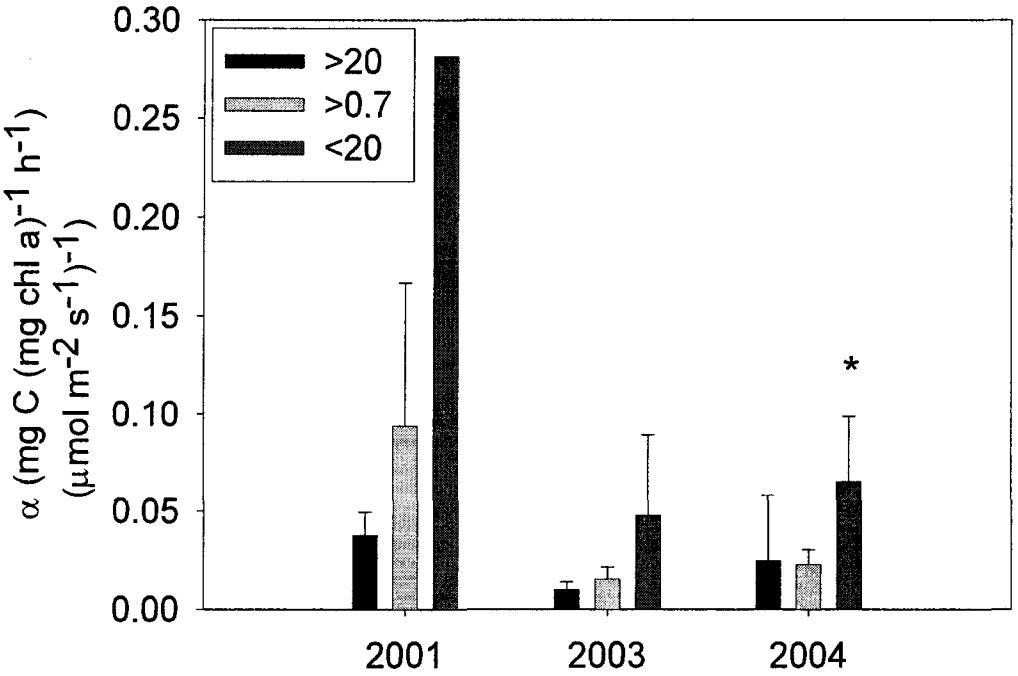


Figure 22. Relationship between maximum photosynthesis (P_m^B) and light limited slope (α).

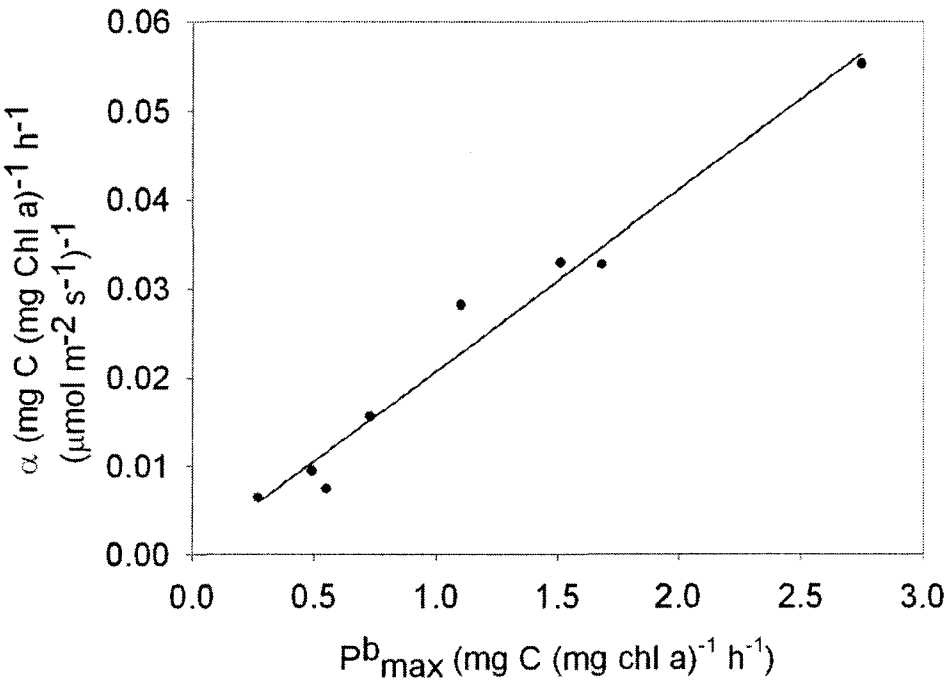


Figure 23. Relationship between percentage of colonial cells (Peloquin, unpublished) and P_m^B. (Circles and squares represent IVARS and JGOFS data, respectively.)

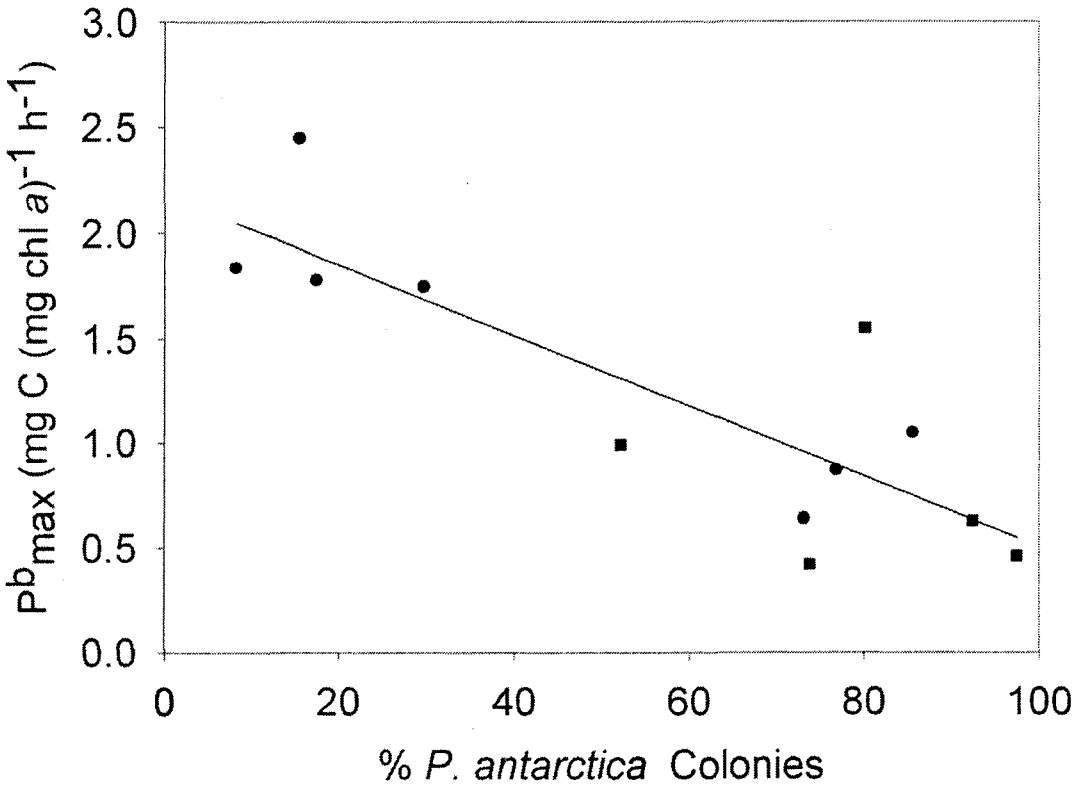


Figure 24. Variation in size fractionated maximal photosynthesis over 16 days with monoculture of *Phaeocystis antarctica* (CCMP 1374). Chlorophyll values are also presented. * denotes a significant difference between the 95% confidence interval of the slopes for the photosynthetic parameter.

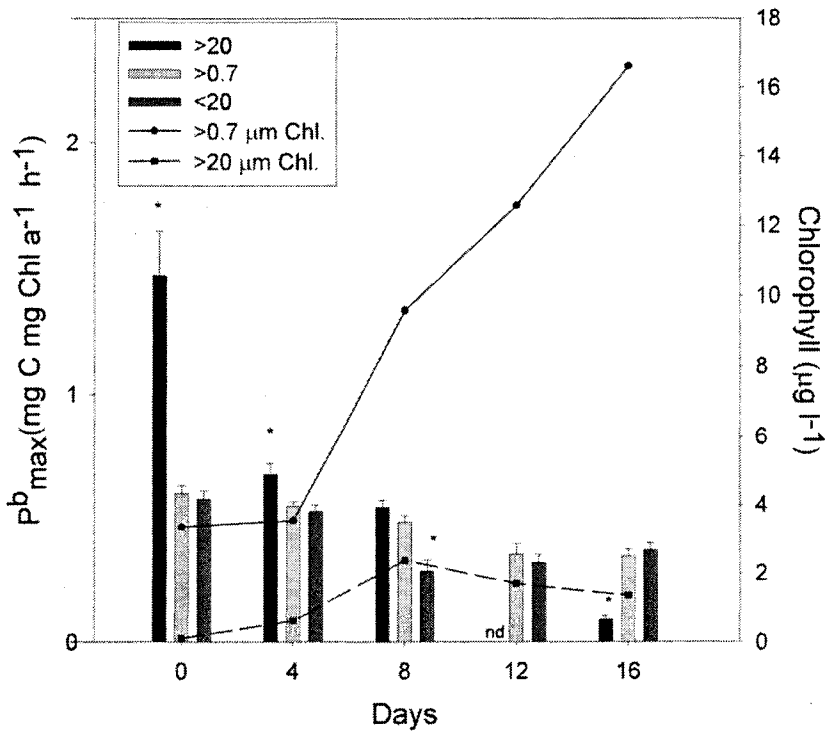
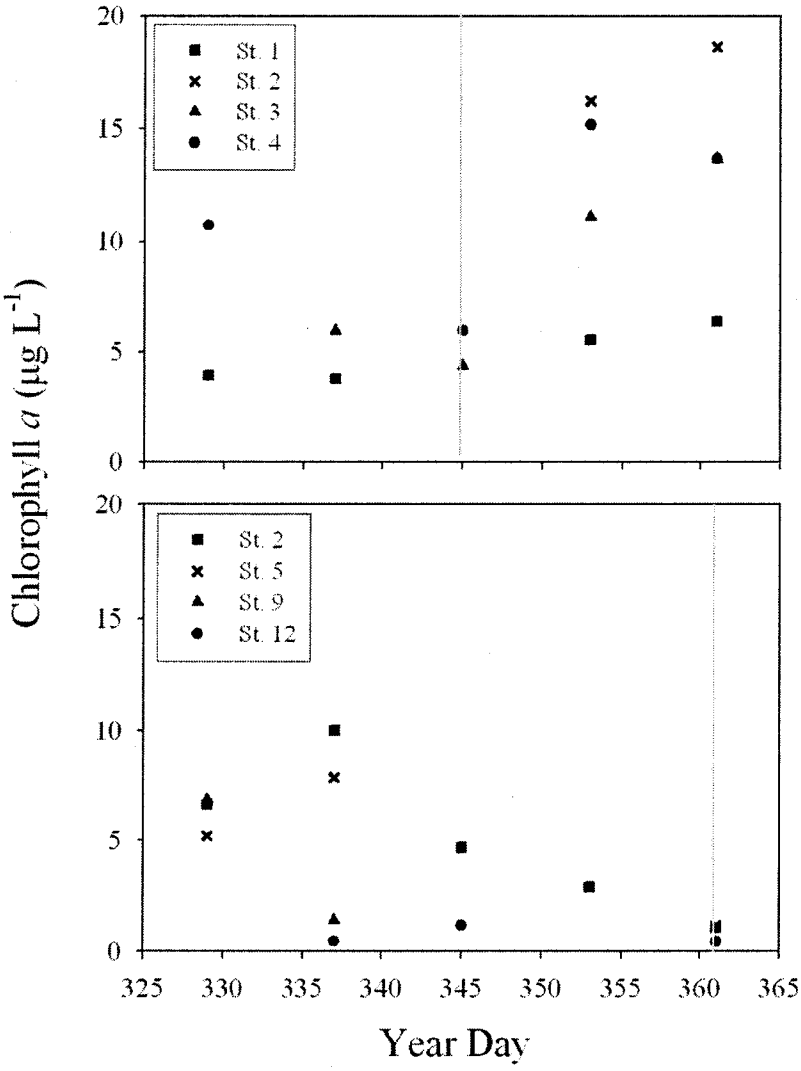


Figure 25. SeaWiFS chlorophyll data output for year day 329-361 during December 2001 and December 2003 (Year day 365= December 31). Gray line represents IVARS sampling date. Data courtesy of Dr. J.A. Peloquin.



Section V. A dual-stain fluorescently labeled algae protocol and an examination of the role of colonial *Phaeocystis antarctica* in the microbial food web of the Ross Sea

ABSTRACT

Morphological defense, in which phytoplankton produce protective external structures or avoid grazers by increasing in size beyond their handling capacity, is speculated to be the strategy of *Phaeocystis antarctica* against grazing by protozoans. Nanoplanktonic (2-20 μm) and microplanktonic (20-200 μm) consumers comprise a significant fraction of total plankton biomass in polar ecosystems. However, the importance of grazing remains uncertain in the Ross Sea, Antarctica. The extensive buildup of phytoplankton biomass in the Ross Sea conflicts with the established view that high rates of herbivory occur in all regions of the Southern Ocean. Large biomass accumulations imply that herbivory and phytoplankton production during austral spring and summer are not balanced. Dilution experiments in previous studies reported that only 25% of all experiments exhibited microzooplankton grazing rates significantly greater than zero. In order to address whether microzooplankton are able to ingest colonial cells of *P. antarctica*, ingestion and clearance rates of single and colonial cells by *Euplotes* (a hypotrich ciliate) were calculated using a novel live-staining fluorescently-labeled algae (FLA) method. Different morphotypes of *P. antarctica* were stained different colors, mixed, and observed inside *Euplotes* to determine their feeding preference. The blue (7-aminocoumarin) (CMAC) stain was used on the colonial fraction (>150 μm), and the green (CMFDA) CellTracker Probe was used on solitary cells. My goal was to compare ingestion rates and presence of *P. antarctica* colonial and solitary cells in *Euplotes* using this dual-staining method. Both morphotypes can be seen inside the food vacuoles of the ciliate, supporting the idea that microzooplankton are capable of ingesting individual cells from the colonial matrix. These results support the conclusion that the microbial

loop plays a larger role in the Ross Sea than once thought and that large *P. antarctica* colonies may actually enter the microbial loop before sedimentation.

INTRODUCTION

Grazing in the Ross Sea

Research in the last several decades has shown that small phytoplankton represent a large portion of primary production in the ocean, and ciliates and other micro-zooplankton have an important role in consuming small primary producers (Kuipers and Witte, 2000; Calbet and Landry, 2004; Dolan et al., 2005). Small protozoa do not produce rapidly sinking fecal pellets; therefore most of the carbon from the primary producers is remineralized in the water column and enters the microbial loop (Azam et al., 1983).

Nanoplanktonic (2-20 μm) and microplanktonic (20-200 μm) consumers comprise a significant fraction of total plankton biomass in polar ecosystems (Garrison et al., 1986). However, the importance of grazing remains uncertain in the Ross Sea, Antarctica. The extensive buildup of phytoplankton biomass in the Ross Sea conflicts with the established view that high rates of herbivory occur in all regions of the Southern Ocean (Caron et al., 2000). Large biomass accumulations imply that herbivory and phytoplankton production during austral spring and summer are not balanced. Temporal offsets, however, could occur, but this has not been shown experimentally (Caron et al., 2000). Smith et al. (1996) suggest that diatoms in the Ross Sea were removed by herbivorous grazing while *P. antarctica* were removed through sinking and aggregation. This causes decoupling of silica and carbon in the surface layer.

This buildup of biomass in the Ross Sea might be due to the ability of phytoplankton to defend against grazers, affecting competitive interactions among species and initiating trophic cascades. Morphological defense, in which a phytoplankton

produces protective external structures or avoids grazers by increasing in size beyond their handling capacity, is speculated to be a strategy for *Phaeocystis* against grazing by protozoans (Jakobsen and Tang, 2002). *Phaeocystis* blooms have been considered 'loopholes' in the microbial loop where their growth and accumulation exceeds losses through predation, sinking and lysis (Irigoien et al., 2005). A 'loophole' is defined as a perturbation such as nutrients and light that allow a bloom species to grow at higher rates than predation losses. Irigoien et al. (2005) argue that the process of colony formation prevents grazers from consuming *Phaeocystis* colonies. Understanding the fate of *P. antarctica* blooms in the Ross Sea is important because *P. antarctica* contributes a majority of primary production, thereby playing an important role in biogeochemical cycling and flux in the Ross Sea. The solitary and colonial forms should have different fates due to the increase in size from solitary cells to large colonies (Schoemann et al., 2005). Colony formation may be a mechanism for the avoidance of grazing pressure and a competitive advantage over solitary cells even when both the colonies and single cells have similar growth rates (Smith et al., 2003). Single cells of *Phaeocystis* can be grazed by ciliates and heterotrophic dinoflagellates (Weisse and Scheffel-Moser, 1990). However, protozoans (Verity and Villareal, 1986) and macrozooplankton (Fryxell and Kendrick, 1988; Verity et al., 1988) cannot consume *Phaeocystis* colonies efficiently. Copepods graze on the colonies (Huntley et al., 1987), but the ingestion rate may be controlled by the physiology of colonies (Estep et al., 1990). The colonial form of *P. antarctica* is relatively ungrazed, with only 13 of 51 dilution experiments yielding significant mortality rates (Caron et al., 2000). Caron et al. (2000) suggest that the extensive blooms in the Ross Sea are due to complex interactions of factors including

phytoplankton size and composition, as well as the low *in situ* temperature. Some preliminary results from dilution experiments in McMurdo Sound and the Ross Sea, Antarctica, however, suggest that microzooplankton grazing can be high during some times of the year (Shields, unpublished). These results suggest that microzooplankton grazing in the Ross Sea should be further explored using novel or alternative methods such as the fluorescently labeled algae technique which may be able to detect lower grazing rates.

Fluorescently labeled algae (FLA) technique

Since microzooplankton are often associated with the colonial matrix of *Phaeocystis*, it is possible that these microzooplankton are grazing upon individual colonial cells rather than the whole colony. In order to address whether microzooplankton are ingesting these colonial cells, ingestion and grazing rates of individual cells must be determined. Ingestion rates and feeding behaviors of protists have been investigated using fluorescently labeled bacteria and algae techniques (Rublee and Gallegos, 1989; Sherr and Sherr, 1983) in which heat-killed cells are fed to grazers. However, many species of protists discriminate or reject these labeled cells (Stoecker, 1988). In order to overcome the discrimination against dead cells and the effects on grazing and ingestion rates, Li et al. (1996) developed a protocol in which CMFDA (5-chloromethyl-fluorescein diacetate) was used to label live cultures of phytoplankton. CMFDA is a vital, biologically inert, green fluorescent stain that allows phytoplankton cells to be visualized inside the grazer's food vacuole for at least 72 hours (Li et al., 1996). The green stain is also easily distinguished from the red chlorophyll fluorescence emitted from the phytoplankton (Li et al., 1996).

Objectives

In order to address whether microzooplankton are able to ingest colonial cells of *P. antarctica*, ingestion and clearance rates of single and colonial cells by *Euplotes* (a hypotrich ciliate) were measured using a novel live-staining fluorescently-labeled algae (FLA) method. The stain, CMFDA, used in Li et al. (1996), was applied to these ingestion rate experiments. Different morphotypes of *P. antarctica* were stained different colors, mixed, and observed inside *Euplotes* to determine their feeding preference. The blue (7-aminocoumarin) (CMAC) stain was used on the colonial fraction (>150 μm) and the green (CMFDA) CellTracker Probe was used on solitary cells. Ingestion rates and presence of *P. antarctica* colonial and solitary cells in *Euplotes* was compared using this dual-staining method.

METHODS

FLA staining

P. antarctica colonial and solitary cells (CCMP 1346) were grown at continuous light and at -1 °C in the laboratory. *Euplotes* (obtained from Dr. David Caron at the University of Southern California) were fed *P. antarctica* for at least two weeks at -1 °C and then starved for 48 hours prior to the experiment. The *P. antarctica* culture was then separated into >150 μm (large colony) and <10 μm (single cell) size fractions using reverse filtration. The reverse filtration method was not 100% efficient, with colonial cells making up 18.8% of the solitary cell treatment and solitary cells making up 5.4% of the colonial cell treatment based on microscopic analyses. Working stocks of CellTracker solutions (CMAC and CMFDA) were made up using dimethyl sulfoxide (DMSO) at 100 μM concentrations. Additional concentrations (5 μM and 0.1 μM) of

CMFDA/CMAC at 0.5, 1, and 2 hours, were also used to determine optimal staining and minimization of background color on the slides with the microzooplankton. The optimal staining procedure was determined as an exposure of *P. antarctica* to CellTracker Green and CellTracker Blue fluorescent stains in f/2 media with 1 μM final concentration for 2 hours in the dark at -1 °C (Figure 26). After staining, the cells were fed to *Euplotes* for 28-h. Four treatments were included in this pilot experiment including (1) a control containing only *Euplotes* and a 0.2 μm filtrate (using an Acrodisc® syringe filter of CMAC and CMFDA), (2) a solitary cell (< 10 μm) treatment stained with CMAC fed to *Euplotes*, (3) a colonial cell (>150 μm) treatment stained with CMFDA fed to *Euplotes*, and (4) a mixed treatment with equal proportions of green stained colonial cells and blue stained solitary cells fed to *Euplotes* (Figures 27a,b). Final concentration was 1.08×10^4 cells mL^{-1} for *P. antarctica* and 1680 cells mL^{-1} of *Euplotes* was added for all treatments. While the concentrations of *Euplotes* were much higher than observed field values, the purpose of this experiment was to determine the success of the method and the ability of *Euplotes* to graze on *P. antarctica* colonial cells, rather than determine *in situ* grazing rates. Two replicates for each treatment were incubated in 50 mL polypropylene centrifuge tubes at -1° C in the dark. Subsamples of 10 mL were taken at 4, 10, 16, 22 and 28-h and filtered onto 0.4 μm black nucleopore filters after preservation with 1% glutaraldehyde. Thirty random *Euplotes* were chosen for observation of Green Fluorescent Inclusions (GFI) and Blue Fluorescent Inclusions (BFI) inside the food vacuoles using epifluorescent microscopy with filters Chroma UV-2A Excitation 340-350 DM400 BA420 for BFI, and Nikon B-2A Excitation 450-490 DM510 BA520 for GFI within 48-h of filtration (Figure 27c,d). Ingestion rates were calculated by assuming

linearity for the initial portion of the uptake curves (FLA protist^{-1} time^{-1}). By dividing the ingestion rate by the concentration of labeled algae, the clearance rate can be calculated (Sherr and Sherr, 1983). Using this linear model, however, assumes the protist is processing food vacuoles in a "conveyor belt" fashion, where food vacuoles have a set lifetime in the ciliate (McManus and Okubo, 1991). Carbon conversion factors for solitary and colonial cells were 3.33 and 13.6 pg C cell $^{-1}$, respectively (Mathot et al., 2000).

RESULTS

Efficiency of FLA staining technique

The CMFDA/CMAC label persisted in the cytoplasm of *P. antarctica* over the 28- h experiment (Figures 27a,b). Background staining of *Euplotes* still occurred. The 1 μM CMFDA/CMAC concentrations maximized fluorescent inclusion visualization in food vacuoles (Figures 27c,d) and minimized background fluorescence. *P. antarctica* fluorescent inclusions were present in all treatments during the duration of the experiment. *Euplotes* did have some background staining and rinsing the cells with filtered seawater could have decreased this effect. Even at 40X magnification, *Euplotes* BFI and GFI could be easily visualized and counted (Figure 27d).

Fluorescent Inclusions

Fluorescent inclusions for all treatments were present in over 50% of the ciliates in all treatments after 4-h (Figure 28). Controls had no inclusions present over the incubation period. The <10 μm had over 80% of *Euplotes* cells with GFI over the 28-h period. The mixture had intermediate values and the >150 μm treatment usually had greater than 50% of the grazers with BFI.

The single cell and mixed treatment (both single cells and colonies) had the highest ingestion of *P. antarctica* throughout the duration of the experiment (Figure 29). As expected, the number of FLA ciliate^{-1} leveled off due to digestion/egestion after a period of time. The colonial *P. antarctica* treatment had less than 2 FLA ciliate^{-1} throughout the experiment. When fed mixed cells, the presence of the BFI and GFI combined were significantly higher than the colonial cell treatment. The grazers in the mixed treatment had a decrease in solitary cell ingestion and ingestion of the colonial form was comparable to the colonial treatment (Figure 30). This suggests that even when *Euplotes* is exposed to both solitary and colonial cells, that *Euplotes* will still graze colonial cells at the same rate showing no preference for cell type or morphology.

Ingestion and clearance rates

There was no significant difference between ingestion rates of *P. antarctica* colonial or solitary cells when comparing the 95% CI of the slopes of the linear regressions (Table 14; Figure 29). The mixed treatment, when *Euplotes* had closer to a natural assemblage of both solitary and colonial cells, had the highest ingestion and clearance rates (ingestion rate 0.26 FLA ciliate^{-1} h^{-1}, clearance rate 0.024 µl ciliate^{-1} h^{-1}) (Figure 30, Table 14). The ingestion rate for solitary (<10 µm) cells decreased from 0.23 to 0.17 FLA ciliate^{-1} h^{-1} in the mixed treatment. The ciliates ingested more single cells than colonial cells, but there was no significant difference between their ingestion rates using comparisons of the 95% Confidence Intervals of the slopes. Grazing of colonial cell carbon was higher than solitary cell carbon in all treatments due to the increased carbon content of colonial cells with carbon ingestion rates highest in the >150 µm treatment.

Colonial cells, therefore, may provide more carbon to microzooplankton than solitary cells even when they are ingested by grazers at a lower rate.

DISCUSSION

FLA Technique

The dual staining FLA technique was an efficient method for determining ingestion rates of *P. antarctica* using *Euplotes* as the grazer. The CMFDA/CMAC label persisted in the cytoplasm of *P. antarctica* over the 28-h experiment, suggesting that this method can be used in long duration grazing experiments. The FLA technique for *Phaeocystis* sp. could provide an increased understanding of the roles of microzooplankton grazing of colonial cells. The use of a dual stain also helped illustrate the preference that a grazer can have for a specific food type. The Green (CMFDA) and Blue (CMAC) dyes were easily visualized in the mixed treatments, but a cell rinse may be beneficial, especially for the green CMFDA dye which, tended to cause more background staining than the CMAC stain. Surrogate food particles that include beads or pigments (McManus and Okubo, 1991) and other FLA/heat-killed techniques will not elucidate how ciliates and other potential grazers could graze colonial *P. antarctica*. Putt (1991) also found that FLA techniques using heat-killed algae underestimated grazing rates. Other experiments that used ciliates with prey stained with CMFDA found that it did not influence the feeding activity of the ciliates (Kamiyama, 2000). They found no difference between the ingestion of labeled and non-labeled prey items. The use of CMAC stain in grazing experiments is infrequent and experiments investigating its affect on feeding activity of ciliates have not been performed. Ciliates did actively graze blue-

labeled, CMAC colonial cells at comparable rates to CMFDA stained cells. Therefore, it is unlikely that the stain had any negative effect on the grazers.

FLA should be considered for further grazing experiments in the Ross Sea and other marine systems. Dolan and McKeon (2005) note that dilution experiments are time consuming and can overestimate grazing rates. They also suggest that in cases where grazing is low as in Caron et al. (2000), that this technique may not be able to quantify these low rates. Another drawback of dilution experiments is that they often have a small number of replicates because the amount of preparation time is large. Finally, Dolan and McKeon (2005) suggest that the grazer community may change, and the grazer concentrations may not be linearly related to the dilution. The drawbacks of FLA experiments are the addition of cultured prey (which increases prey density), and that the green CMFDA might be confused with the autofluorescence of heterotrophic dinoflagellates (Li et al., 1996). A direct comparison of the dilution and FLA method could be a useful tool in assessing grazing rates in many marine systems.

Ingestion and clearance rates

Rose and Caron (2007) describe how there is a constraint on microzooplankton growth rates relative to the effects of temperature on phytoplankton growth rates. These decreased growth rates (Q_{10}=3.75 for herbivores) compared to phytoplankton (Q_{10}=1.88) might explain the large *P. antarctica* blooms in the Ross Sea. The effect of temperature on *Euplotes* and other microzooplankton ingestion rates has not been explored in detail in the Ross Sea. However, microzooplankton grazing rates have been observed to exhibit a positive relationship with temperature (Verity et al., 2000). Low temperature can affect organisms by decreasing chemical reactions and through altering viscosity and diffusion

in the marine environment (Begon et al., 1986). Encounter rates and digestion can also increase with temperature. Kamiyama (2000) found higher ingestion rates for another aloricate ciliate (*Loboea strobila)* of 1.83 cells ciliate^{-1} h^{-1} on *Heterocapsa triquetra* (23.9 μm X 17.3 μm) at 20 °C. Ingestion rates in this study were much lower. The highest ingestion rate measured in our experiment was 0.26 FLA ciliate^{-1} h^{-1}. A Q_{10} value of 2.8 is an average for zooplankton rate processes (Hansen et al., 1997). Using a Q_{10} value of 2.5 (Caron et al., 1990) that included both the effects of prey concentration and temperature, *Loboea strobila* would have had an ingestion rate of 0.26 FLA grazer^{-1} h^{-1}, identical to what was calculated for our highest ingestion rate in the mixed treatment. *Heterocapsa triquetra* is also much larger than *P. antarctica* solitary cells and is a harmful algal species. While *Loboea* and *Euplotes* may not be directly comparable, our ingestion rates calculated during this experiment are what would be calculated using Q_{10} values alone. *Euplotes vannus* has been observed to have an ingestion rate 2-54 cells ciliate^{-1} h^{-1} (Premke and Arndt, 2000). Q_{10} values calculated using the lower values of that range also estimate 0.29 FLA^{-1} ciliate^{-1} h^{-1} with a maximum in that range of 7.3.

It is surprising that *Euplotes* would graze on *P. antarctica* at all, as Rousseau et al. (2000) concluded that during large *Phaeocystis* blooms in the North Sea that 75% of the carbon ingested by zooplankton was from diatoms even when they represented only 30% of the whole assemblage. Future experiments in the field when diatoms are also present along with *P. antarctica* will assess whether ciliates utilize it as a preferred food source. Ciliates, however, have been shown to be important grazers of *Phaeocystis* in some marine systems (Admiraal and Venekamp, 1986; Weisse and Scheffel-Möser, 1990; Tang

et al., 2001). Ciliates generally prefer nanophytoplankton under 20 μm (Bernard and Rassoulzadegan, 1990; Hansen, 1992).

Ingestion rates could have also been underestimated during our study. Grazers and the labeled cells were incubated in the dark to minimize light effects on chemical reactions (Li et al., 1996). Complete darkness during our incubations could have lowered the rates since light can enhance growth rates and feeding efficiency of the grazer (Strom, 2001). It is recommended that future field experiments should be performed under *in situ* light conditions since the dye did not fade quickly over the 28-h period. In order to get more data in the linear portion of the uptake curve, shorter experiments (15 h) with more time periods would be recommended. Slides also must be examined quickly since our slides began to fade after inspection one week after initial counts were made.

Colonial *P. antarctica* ingestion

Colony formation may be a strategy of *Phaeocystis* for avoiding grazing pressure (e.g., Jakobsen and Tang, 2002). Microzooplankton structure phytoplankton communities during *P. globosa* blooms in the North Sea (Stelfox-Widdicome et al., 2004). While ciliates and heterotrophic dinoflagellates show positive growth and active ingestion of solitary cells (Weisse and Schefel Moser, 1990; Tang et al., 2001), our preliminary results suggest that *P. antarctica* colonial cells actually may represent more of the daily carbon ingestion by microzooplankton than solitary cells, especially during large blooms. For example, since colonial cells contain about four times more carbon than solitary cells, the highest carbon ingestion rate by *Euplotes* is in the >150 μm treatment (2.3 pg C ciliate^{-1} h^{-1}). Microzooplankton have been observed to actively move in and out of colonies (Shields, unpublished observations). Hamm (2000) also suggests that smaller

organisms may be able to enter the colonies the same way other organisms such as rotifers penetrate and feed on *Volvox* colonies. Field observations using this technique would provide estimates of the magnitude of microzooplankton utilization of colonial *P. antarctica* as a food source. The grazer community would be more diverse, and the grazers would have other species of phytoplankton to preferentially ingest.

Clearance Rates

Clearance rates were highest in the mixed treatment (0.024 μL ciliate^{-1} h^{-1}), though there is uncertainty associated with our measurements due to small sample sizes used in the linear regressions. In addition, these clearances rate calculations will not apply to surface grazers in the water column. Clearance rates for ciliates rarely exceed 10 μL h^{-1} (Capriulo et al., 1991). Rates higher than this are usually in warm, low chlorophyll waters. Pitta et al. (2001) found lower clearance rates of 1 μL ciliate^{-1} h^{-1} based on food vacuole content. Ciliates are able to discriminate prey on different prey characteristics besides just prey volume and are not simple mechanical feeders (Stoecker, 1988; Verity, 1991; Li et al. 1996). In addition, *Euplotes* is a benthic ciliate and is associated with surfaces while it scavenges particles (Wilks and Sleigh 1998), and clearance rates may be underestimated because in the natural environment they would be encountering prey in greater numbers. The high feeding rates on *P. antarctica* colonial cells could be due to colonies representing a benthic like environment that is analogous to the marine snow that *Euplotes* graze in the natural environment. The ciliates may also be grazing upon bacteria associated with the *Phaeocystis* colonies, but we have no estimates of the ingestion rates on this prey.

Field experiments

Additional field experiments using this technique were planned in McMurdo Sound over the past two years to estimate the relative ingestion rates of colonial and solitary *P. antarctica* by a natural grazer assemblage. Field experiments when natural grazers were abundant along with other food sources, would allow for further observations of the ingestion rates and the magnitude of colonial *P. antarctica* grazing. But, due to the low abundances of microzooplankton in January 2006 and 2007, we were not able to get the high abundances that would be needed for this experiment.

Microzooplankton experiments were performed in parallel to these FLA attempts (Tang and Shields, unpublished). During late January and early February microzooplankton dilution experiments resulted in higher grazing rates in McMurdo Sound compared to Caron et al. (2000). FLA combined with dilution experiments in the Ross Sea would determine the magnitude of herbivorous grazing where *P.antarctica* forms large phytoplankton blooms in open polynyas.

Conclusions

Due to the critical role of the morphological form of *Phaeocystis antarctica* in carbon transformations and food web dynamics, it is important to understand the controls of the various life stages of the species. Unlike *P. globosa*, the evolution of the life history strategy involving the transition between solitary and colonial cells may not be due to grazing. This is supported by observations that as colonial and solitary cells are being grazed at the same rate in these FLA experiments. These results also suggest that the microbial loop may play a significant role in the Ross Sea and that large colonies may actually enter the microbial food web before sedimentation. *P. antarctica* colonies were

not observed to be in high abundance in our sediment trap collection during 2001-2004. The rapid export of *P. antarctica* in the Ross Sea prior to entering the microbial food web, therefore, may not reflect all years and locations in the Ross Sea.

While it is widely believed that mesozooplankton and microzooplankton do not effectively graze colonial cells, the results from this study show that some grazers may ingest single and colonial cells at the same rate. Little is known about rates of grazing by nano- (2-20 μm) and microzooplankton (20-200 μm) on phytoplankton in the Ross Sea. Diverse assemblages of ciliates, heterotrophic dinoflagellates, and choanoflagellates have all been observed in other studies. The grazer used in this experiment is benthic and associates with marine snow and other aggregates, therefore additional studies using planktonic microzooplankton will assess the ability of other grazers to feed on colonial *P. antarctica* cells. These future experiments looking at the utilization of *P. antarctica* as a food source by micro and mesograzers may change our view of the importance of the Ross Sea microbial food web.

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Table 14. Calculated ingestion and clearance rates for *Euplotes*. Ingestion rates were calculated by plotting FLA cilate^{-1} vs. time (hour) and a regression of the linear portion of the uptake curve was performed. Clearance rates were calculated by dividing the ingestion rate by the concentration of FLA per µL added (Sherr and Sherr, 1993).

Treatment	**Ingestion Rate (FLA ciliate^{-1} h^{-1})**	**Clearance Rate (µL ciliate^{-1} h^{-1})**
Control	0	0
>150 µm	0.17 R^2= 67.8%; p=0.044	0.015
<10 µm	0.23 R^2= 91.4%; p=<0.0001	0.021
Mix	0.26 R^2=93.7%; p=<0.0001	0.024
Mix <150 µm	0.11 R^2=84.2%;p=0.004	0.009
Mix <10 µm	0.17 R^2=85.9%;p=0.003	0.016

Figure 26. Diagram of fluorescently labeled Algae (FLA) methodology using a dual staining method.

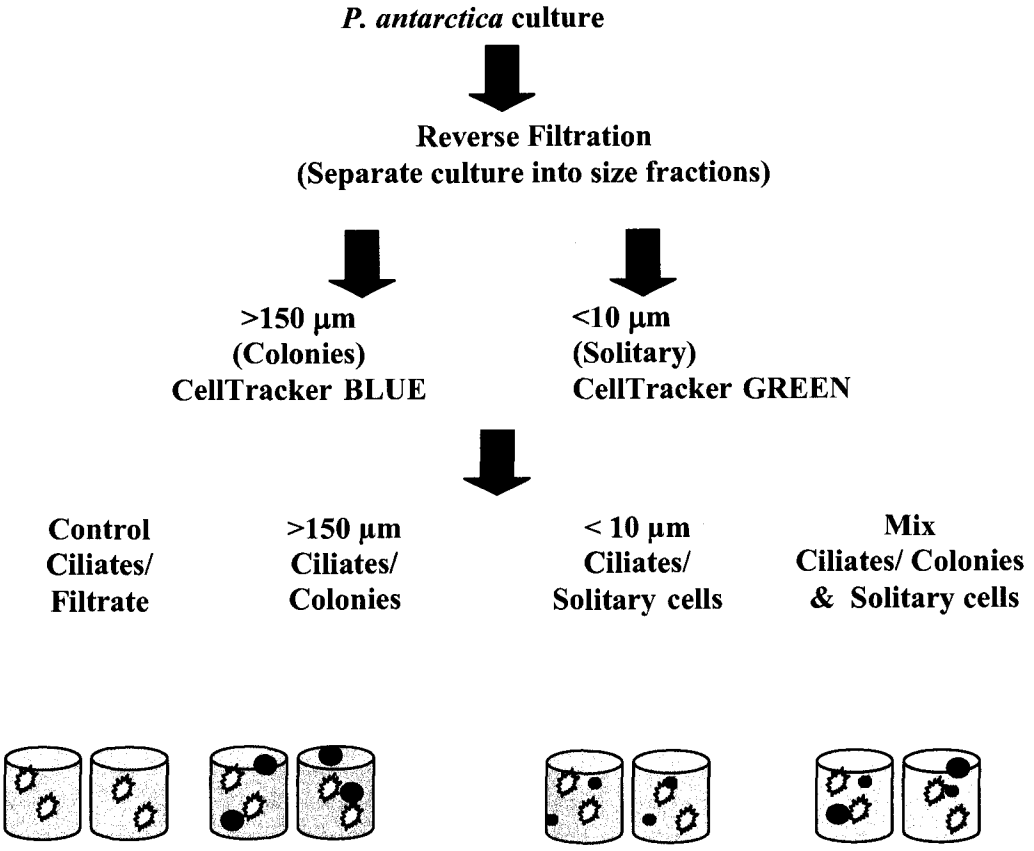
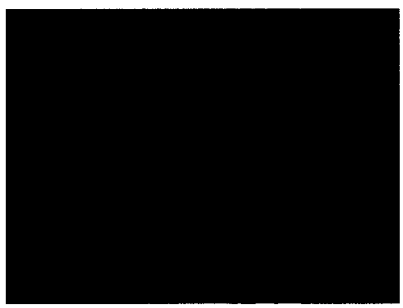


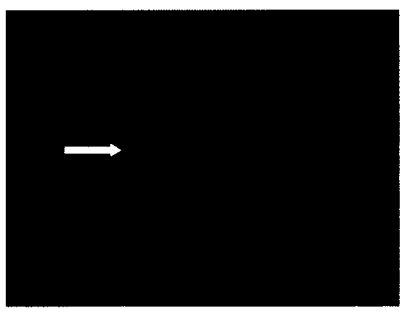
Figure 27 a) *P. antarctica* solitary cells after stained with 1 μM CMFDA (Green) for two hours under epifluorescent microscopy (1000X). b) *P. antarctica* colonial cells after stained with 1 μM CMAC (Blue) for 2 hours under epifluorescent microscopy (1000X). c) *P. antarctica* solitary cells after stained with 1 μM CMFDA (Green) ingested by *Euplotes* under epifluorescent microscopy (1000X). Arrow points to ingested cells inside ciliate. d) *P. antarctica* cells after stained with 1 μM CMFDA (Green) ingested by *Euplotes* under epifluorescent microscopy (400x).

a)

b)



c)



d)

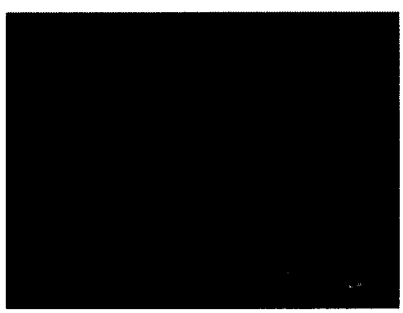


Figure 28. Presence of *P. antarctica* fluorescent inclusions inside *Euplotes* food vacuoles. Error bars represent averages $\pm$ s.e.m. (n=2)

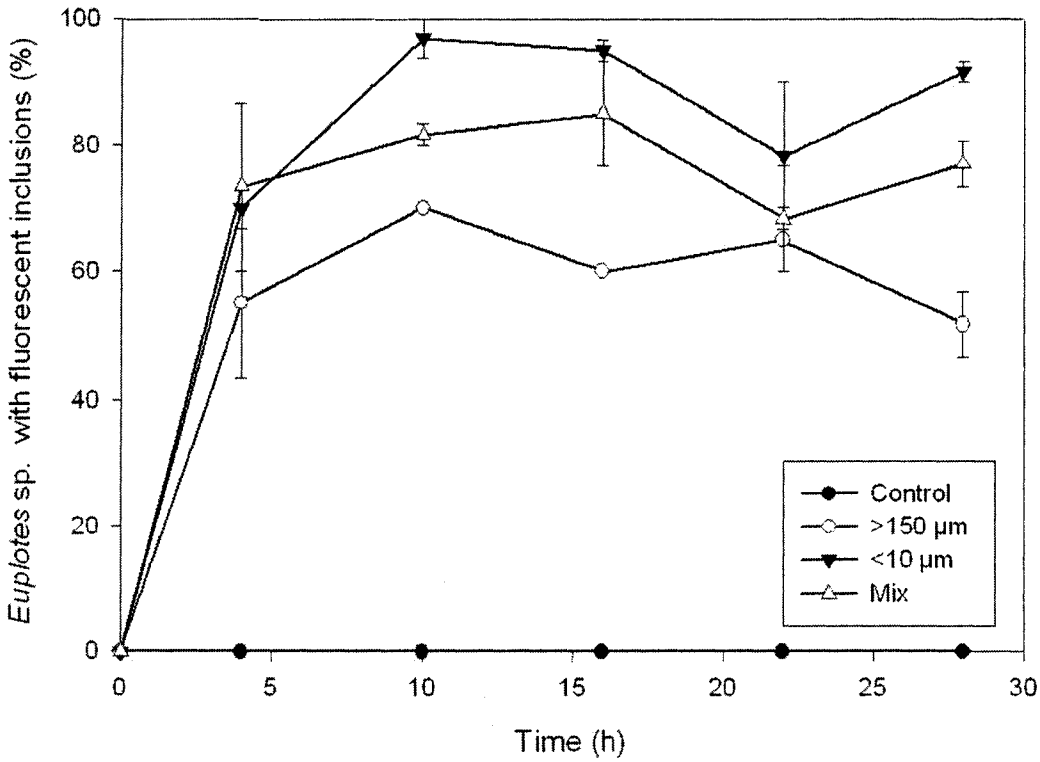


Figure 29. The average number of FLA inclusions per ciliate in each treatment. Error bars represent averages $\pm$ s.e.m. (n=2)

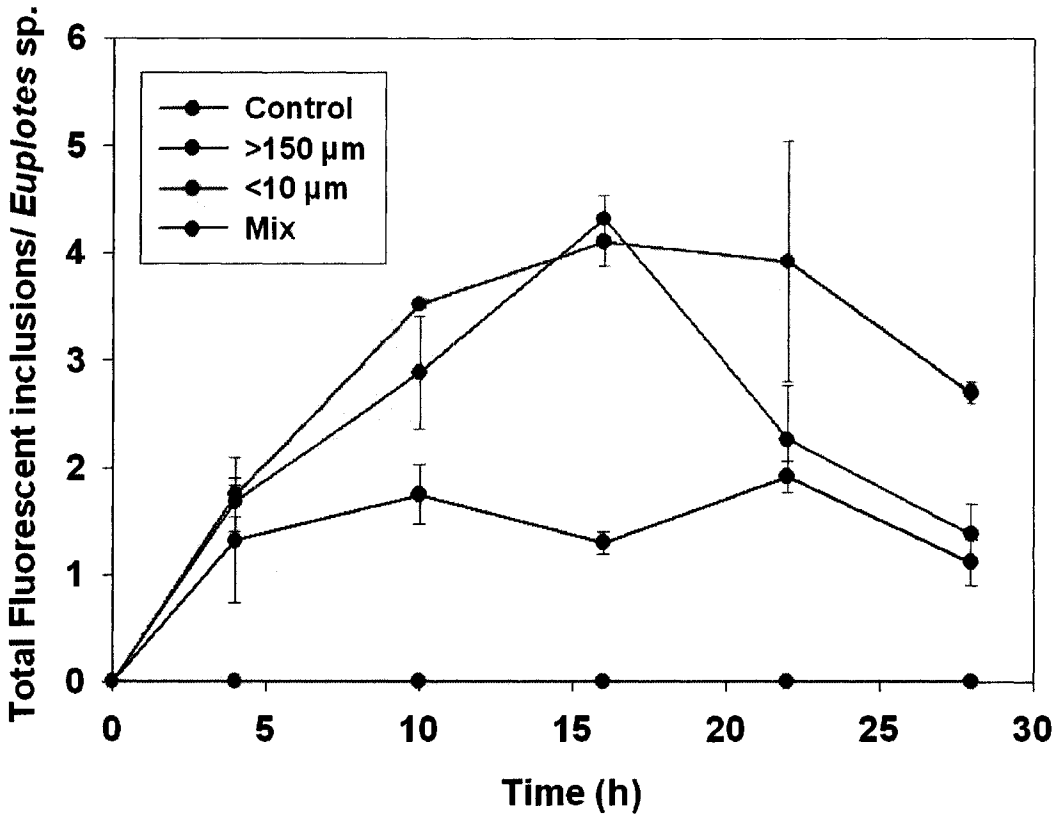
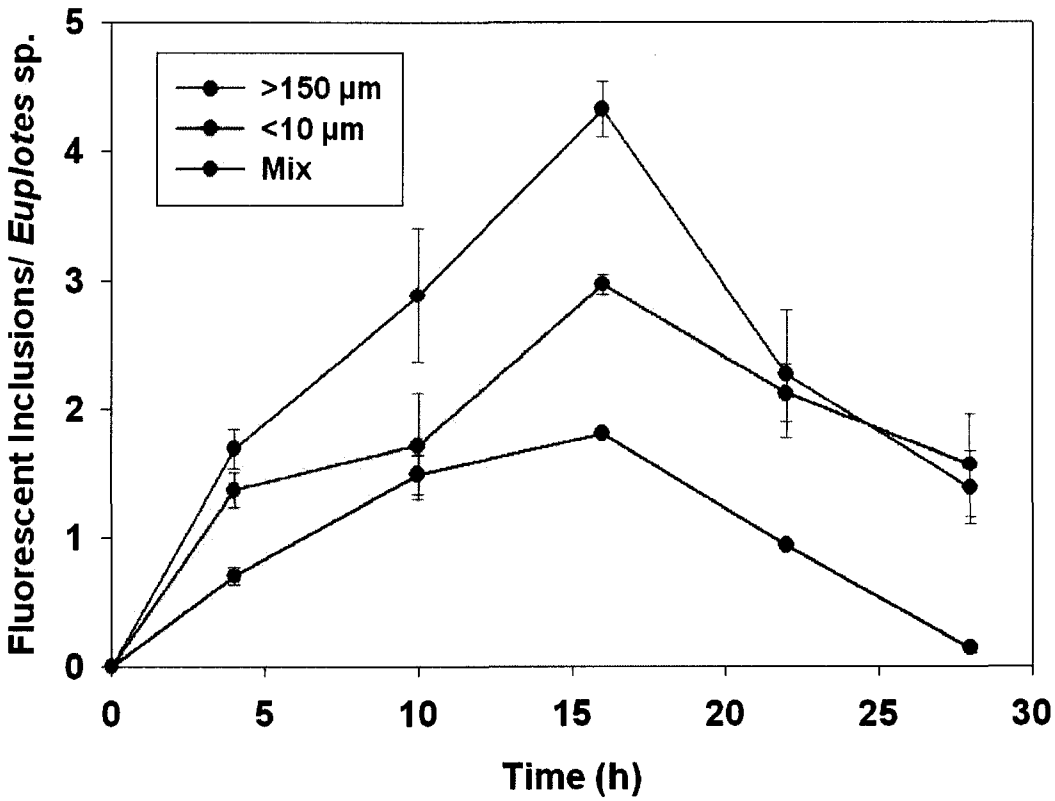


Figure 30. The average number of inclusions per ciliate in the mixed treatment (ciliates were fed both single and colonial cells). Error bars represent averages ± s.e.m. (n=2)



Section VI. Synthesis and Conclusions

The primary objective of this dissertation was to study three interrelated processes of the biological pump, which is the principal biological regulator of ocean-atmosphere carbon cycling, of the Ross Sea: primary production, export, and the role of grazers in the acceleration of carbon flux to depth. As part of the Interannual Variability of the Antarctic-Ross Sea program, the interannual variability in these processes was also explored.

Carbon Production and Nutrient Budgets

As shown in Section I, nutrient data were collected from within transects of largely ice free regions, and simple, one dimensional nutrient budgets were made using *in situ* nitrogen and silicon concentrations. Using these budgets, phytoplankton carbon production and export were calculated. While *Phaeocystis antarctica* was thought to primarily dominate the southeastern sector of the Ross Sea and diatoms dominate the southwestern sector, interannual variations showed significant variability in both bloom composition (diatoms or *P. antarctica*) and in magnitude, interannually and seasonally. During February 2004, a large secondary bloom of diatoms occurred due to water mass intrusions that delivered micronutrients. This bloom was greater in magnitude than the February climatology and historical observations and summer uptake of nitrogen had an 8-fold increase compared to the other years.

Primary controls of phytoplankton blooms

Section II included a synthesis of the data from Section I. Principal components analysis was used to ascertain the main factors in December bloom development in the Ross Sea. This analysis was critical in examining patterns in the large IVARS data set. Through visualization of the loadings and scores of the principal components, the

primary controls of biomass and organic matter were seasonality combined with phytoplankton community composition and temperature. Further regression analysis of sea surface nitrate and temperature suggested a positive relationship of temperature and nitrate removal in the surface waters of the Ross Sea. Data from the United States Joint Global Ocean Flux (JGOFS) program was combined with the IVARS data set. Since temperature and nitrate had a significant linear relationship, the Goes et al. (1999) model was used using data that can also be predicted using remote satellite measurements, chlorophyll and temperature. Our model was successful in predicting sea surface nitrate concentrations from temperature and chlorophyll in the Ross Sea and may be utilized as a tool in predicting new production (from nitrate) and export at a larger scale than ship measurements alone.

Vertical Flux

In order to understand the efficiency of the biological pump during IVARS, trap collections were examined from 2003-2004 (~ 40 days) that was dominated by a large *P. antarctica* colonial bloom in late December to mid-January and a secondary diatom bloom greater in magnitude from late January to our sediment trap retrievals in February 2004. This trap material and its biogenic composition were compared to December 2004-2005, where a large diatom bloom occurred in December. Biological uptake of nitrogen during January 2005 was minimal and export compared to January 2004 was expected to be minimal. While this short trap deployment only allowed for a glance at the export for both seasons, fecal pellet carbon flux represented a large percentage of flux for both years which, suggests that mesozooplankton grazers were actively grazing and packaging phytoplankton in quickly sinking fecal pellets. One major conclusion from this section

was that ovoid green and brown pellets represented flux at > 100% during certain intervals of trap material collection. Herbivorous grazing of phytoplankton, therefore, should be further explored due to the role it would have on vertical export and increasing efficiency of the biological pump.

Photosynthetic parameters of colonial and solitary *P. antarctica*

Phaeocystis antarctica is the main source of primary production and vertical flux in the Ross Sea and understanding colony formation is critical in the determination of biogeochemical cycling. Section IV examined the role that morphology (colonial vs. solitary) plays in maximum photosynthetic rates. Experiments from IVARS were combined with culture work to describe how bloom stage affects photosynthesis. During December 2001, maximum photosynthetic rates of the bulk phytoplankton assemblage were significant compared to the other years. Also, during 2003-2004 solitary cells had significantly higher photosynthetic rates than colonial cells. While the conclusion that solitary cells are more efficient in photosynthesis during all times, further examination of satellite chlorophyll measurements showed that 2001-2003 and 2003-2004 different in the bloom stage of *P. antarctica*. During December 2001, our sample collection was during the exponential phase of the *P. antarctica* bloom (chlorophyll continued to increase after our sample collection), while in December 2003, the bloom of colonial *P. antarctica* was in decline. Iron limitation was observed (Peloquin, 2005) which may have driven the low maximum photosynthetic rate of colonial *P. antarctica*. The laboratory studies over a 16-d period suggested that the maximum photosynthesis of the colonial form of *P. antarctica* was significantly higher than solitary cells during the exponential phase of the bloom. After the 16-d period (where nutrients were limiting),

maximum photosynthesis of the solitary cells was significantly higher than the colonial cells. These are the first results to show that bloom stage affects the maximum photosynthetic rates of the different morphological forms of *P. antarctica* and are the first to describe how colonial *P. antarctica* may grow and form large colonies in the Ross Sea earlier than diatoms.

Microzooplankton grazing on colonial *P. antarctica*

A dual-staining procedure to examine the ingestion rates of colonial and solitary *P. antarctica* was developed in Section V. The combination of two probes (CMFDA and CMFDA) was efficient in allowing for the visualization of the two morphological forms of *P. antarctica* in *Euplotes*. The colonial cells of *P. antarctica* contain over four times more carbon than the solitary cells. Therefore, even though *Euplotes* ingested solitary cells at a higher rate, carbon ingestion rates of colonial *P. antarctica* were higher. This is the first experimental result suggesting that *Euplotes* and possibly other microzooplankton may be capable of grazing upon *P. antarctica* colonial cells in the colonial matrix. This procedure would allow for the further examination of the actual grazing rates of *P. antarctica* by natural grazers in the Ross Sea and would be more efficient than analyzing microzooplankton grazing through dilution experiments which, are time consuming and are not useful in measuring grazing in areas with low grazing rates, such as the Ross Sea.

Future directions

A combination of all of these sections allows for further insight into the regulation of the biological pump by primary producers. While primary producers appear to be driven by seasonal factors such as temperature and water mass intrusions, other variables

not measured in our study such as iron should be further explored to make definitive conclusions on whether sea surface temperature controls phytoplankton composition and export. This study raised more questions than it answered. Several important questions come out of this project, and the IVARS project including the role that water mass intrusions play in driving secondary blooms in the Ross Sea and which parameter (temperature or iron) is affecting the bloom composition and magnitude. By isolating specific mechanisms that control phytoplankton growth, accumulation and loss through grazing and sedimentation, we will be able to further predict how future changes in climate or increased water mass intrusions will affect the efficiency of the biological pump and carbon sequestration in the Ross Sea.

Appendices

Appendix A. December 2001 and February 2002 scores from Principal Components Analysis of IVARS data set located at www.vims.edu/bio/ivars.

Year	Season	Longitude (°E)	Scores PC1	Scores PC2	Scores PC3
2001	Dec	173.2	-0.6	-2.7	-0.8
2001	Dec	173.2	-0.4	-2.8	-0.6
2001	Dec	173.2	-1.8	-3.0	-1.5
2001	Dec	175.6	0.2	-3.7	-0.7
2001	Dec	175.6	-0.2	-3.7	-1.1
2001	Dec	175.6	-2.0	-2.8	-1.2
2001	Dec	179.2	-1.0	-2.7	-1.0
2001	Dec	179.2	0.5	-3.9	-1.3
2001	Dec	179.2	-1.2	-3.2	-1.3
2001	Dec	-178.8	-2.8	-1.8	-1.8
2001	Dec	-178.0	-0.2	-2.0	-1.8
2001	Dec	-178.0	0.5	-2.6	-2.3
2001	Dec	-176.7	-0.5	-2.1	-0.7
2001	Dec	-176.7	-0.2	-2.6	-1.2
2001	Dec	-176.7	-2.5	-1.7	-1.6
2002	Feb	171.8	-0.2	1.1	-1.1
2002	Feb	171.8	0.8	0.7	-1.2
2002	Feb	175.5	0.5	0.4	-1.3
2002	Feb	175.5	0.2	0.3	-1.2
2002	Feb	175.5	0.7	0.0	-1.4
2002	Feb	176.7	0.3	0.7	-0.8
2002	Feb	176.7	1.1	-0.1	-0.8
2002	Feb	176.7	-1.1	-0.7	-0.4
2002	Feb	-178.8	-1.2	1.4	-1.8
2002	Feb	-178.8	-1.7	1.3	-1.9
2002	Feb	-178.8	-0.3	0.5	-1.8
2002	Feb	-178.0	-1.0	1.5	-1.4
2002	Feb	-178.0	-0.7	1.4	-1.4
2002	Feb	-178.0	-1.2	1.2	-1.2

Appendix B. December 2003 and February 2004 scores from Principal Components Analysis of IVARS data set located at www.vims.edu/bio/ivars.

Year	Season	Longitude (°E)	Scores PC1	Scores PC2	Scores PC3
2003	Dec	172.8	0.8	-0.1	-0.6
2003	Dec	172.8	1.2	-1.2	0.1
2003	Dec	173.8	0.8	-3.3	0.0
2003	Dec	173.8	-2.0	-2.9	-1.2
2003	Dec	174.8	2.1	-1.1	-0.8
2003	Dec	174.8	0.6	-3.4	1.5
2003	Dec	174.9	2.0	-1.6	-1.1
2003	Dec	174.9	2.2	-1.4	-0.6
2003	Dec	174.9	-2.6	-2.4	-1.4
2003	Dec	176.9	-0.9	0.7	0.2
2003	Dec	176.9	1.8	-2.4	-1.2
2003	Dec	176.9	-2.4	-2.4	-1.2
2003	Dec	177.9	0.8	0.6	-1.2
2003	Dec	177.9	-0.9	-0.3	-2.1
2003	Dec	177.9	-3.6	-1.4	-0.8
2003	Dec	179.0	-1.2	0.8	-0.7
2003	Dec	179.0	-1.4	-1.4	-1.4
2004	Feb	-177.9	3.3	3.4	-1.8
2004	Feb	-177.9	2.2	2.7	-1.4
2004	Feb	-177.9	-1.1	2.5	-1.7
2004	Feb	-179.0	0.7	2.7	-1.5
2004	Feb	-179.0	0.8	2.5	-1.5
2004	Feb	-179.0	-0.6	2.0	-2.1
2004	Feb	179.0	5.5	1.8	-2.5
2004	Feb	179.0	6.3	0.1	-3.9
2004	Feb	177.9	6.5	1.4	-2.5
2004	Feb	177.9	5.4	1.3	-1.1
2004	Feb	177.9	-2.6	0.5	-1.4
2004	Feb	176.9	6.3	2.0	-2.6
2004	Feb	176.9	6.3	1.7	-2.7
2004	Feb	176.9	-1.2	0.9	-1.3
2004	Feb	175.9	3.6	1.3	-5.2
2004	Feb	175.9	5.4	3.5	-2.1
2004	Feb	175.9	-2.8	1.0	-2.4
2004	Feb	174.8	-2.7	1.8	-1.0
2004	Feb	172.8	6.1	2.1	-1.5
2004	Feb	172.8	5.1	2.0	-1.9
2004	Feb	172.8	-3.9	0.3	-1.3

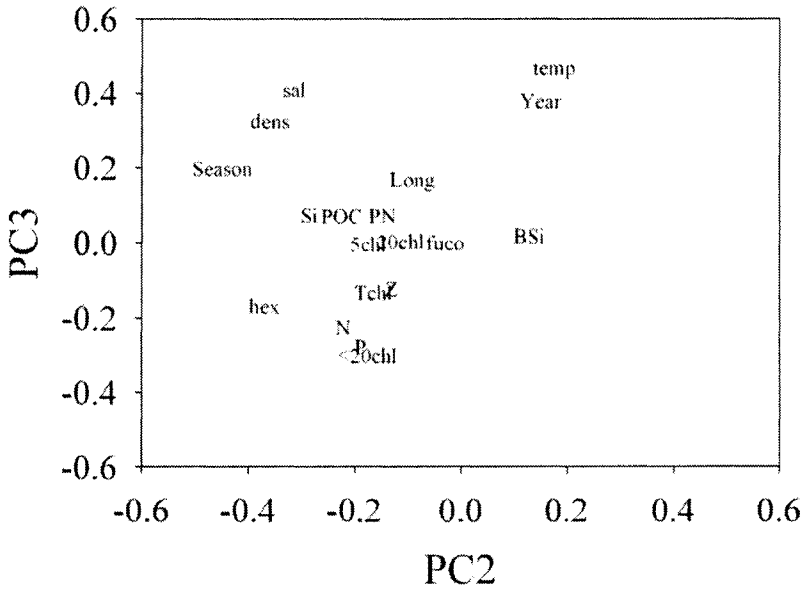
Appendix C. December 2004and February 2005 scores from Principal Components Analysis of IVARS data set located at www.vims.edu/bio/ivars.

Year	Season	Longitude (°E)	Scores PC1	Scores PC2	Scores PC3
2004	Dec	170.8	0.7	1.1	2.8
2004	Dec	170.8	0.1	-1.6	1.2
2004	Dec	170.8	-0.4	-2.5	0.1
2004	Dec	172.8	5.7	-0.8	2.8
2004	Dec	172.8	0.8	-2.5	0.9
2004	Dec	173.8	-1.0	0.8	3.0
2004	Dec	173.8	-1.1	0.6	2.5
2004	Dec	174.8	-1.8	0.7	2.7
2004	Dec	174.8	-1.8	0.3	2.3
2004	Dec	174.8	-2.3	-1.4	0.7
2004	Dec	175.9	7.8	-1.0	2.8
2004	Dec	175.9	4.6	-1.2	2.2
2004	Dec	176.9	3.3	-1.6	1.9
2004	Dec	176.9	3.5	-1.9	1.2
2004	Dec	176.9	-1.1	-2.8	-0.3
2004	Dec	177.9	5.0	-0.4	2.5
2004	Dec	177.9	1.3	-0.7	1.6
2004	Dec	177.9	0.3	-2.6	-0.4
2004	Dec	179.0	4.5	-0.6	1.8
2004	Dec	179.0	2.9	-1.4	1.6
2004	Dec	179.0	-3.1	-1.5	-0.4
2004	Dec	180.0	4.6	-0.5	1.1
2004	Dec	180.0	2.6	-0.9	1.1
2004	Dec	180.0	-2.8	-1.3	0.1
2004	Dec	-178.9	3.3	-0.7	0.8
2004	Dec	-178.9	2.1	-0.8	0.7
2004	Dec	-178.9	-3.2	-0.9	-0.5
2004	Dec	-178.9	4.8	-0.6	1.4
2004	Dec	-178.9	-0.3	-1.2	-1.0
2004	Dec	-180.0	2.7	-0.2	1.3
2004	Dec	-180.0	1.6	-0.3	0.8
2004	Dec	-180.0	-3.6	-1.3	-0.5
2004	Dec	176.9	3.8	-1.1	2.9
2004	Dec	176.9	-2.1	-2.6	0.0
2004	Dec	175.9	0.6	-0.2	2.7
2004	Dec	175.9	0.7	0.0	2.6
2004	Dec	175.9	-2.7	-1.5	0.0
2004	Dec	174.7	0.8	0.6	2.4
2004	Dec	174.7	0.5	-0.4	2.1
2004	Dec	174.7	-2.7	-1.0	0.5
2004	Dec	173.8	-0.1	0.4	2.3
2004	Dec	173.8	-0.1	-0.3	1.9
2004	Dec	173.8	-3.2	-1.2	-0.2
2004	Dec	172.8	2.6	0.4	3.4
2004	Dec	172.8	2.5	0.1	3.8

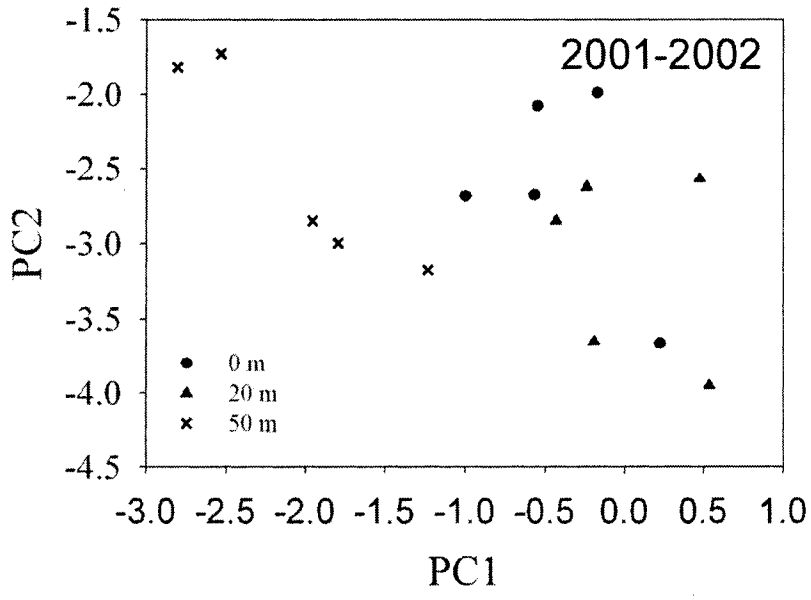
Appendix C, cont. December 2004and February 2005 scores from Principal Components Analysis of IVARS data set located at www.vims.edu/bio/ivars.

Year	Season	Longitude (°E)	Scores PC1	Scores PC2	Scores PC3
2004	Dec	172.8	-1.8	-2.3	0.8
2004	Dec	171.8	3.3	-0.2	2.9
2004	Dec	171.8	3.4	-0.3	2.4
2004	Dec	171.8	-3.7	-1	0.4
2005	Feb	171.8	-2.2	2	0.6
2005	Feb	171.8	-2.5	1.7	0.4
2005	Feb	171.8	-3.4	0.7	-0.2
2005	Feb	172.8	-1.9	1.8	1.6
2005	Feb	172.8	-3.2	0.9	1
2005	Feb	173.8	-1.6	2.5	1.5
2005	Feb	173.8	-1.5	2.1	1.5
2005	Feb	173.8	-3.6	0.2	0.3
2005	Feb	174.8	-1.7	1.8	1.4
2005	Feb	174.8	-1.6	1.6	1.3
2005	Feb	174.8	-2.5	1.3	1.2
2005	Feb	175.9	-2.6	1.8	1
2005	Feb	176.9	-2.4	1.5	1.3
2005	Feb	176.9	-2.6	1.2	1.3
2005	Feb	177.9	-2.1	2	0.7
2005	Feb	177.9	-2.4	2	0.6
2005	Feb	177.9	-2.8	1.6	0.5
2005	Feb	179	-2.3	1.9	0.5
2005	Feb	179	-2.4	2	0.4
2005	Feb	180	-2.2	1.9	1.1
2005	Feb	180	-2.4	2	1
2005	Feb	180	-2.9	1.7	0.8
2005	Feb	-178.9	-1.6	1.9	0.5
2005	Feb	-178.9	-1.9	2.2	0.5
2005	Feb	-178.9	-2.7	1.8	0
2005	Feb	-177.9	-1	2.6	-0.6
2005	Feb	-177.9	-1.1	2.5	-0.7
2005	Feb	-177.9	-1.8	2	-0.5
2005	Feb	175.9	-1	2	1.2
2005	Feb	175.9	-3.4	0.4	0.3
2005	Feb	174.8	-0.7	2.7	1.5
2005	Feb	174.8	-1.2	2.4	1.2
2005	Feb	174.8	-2.3	1.1	0.2

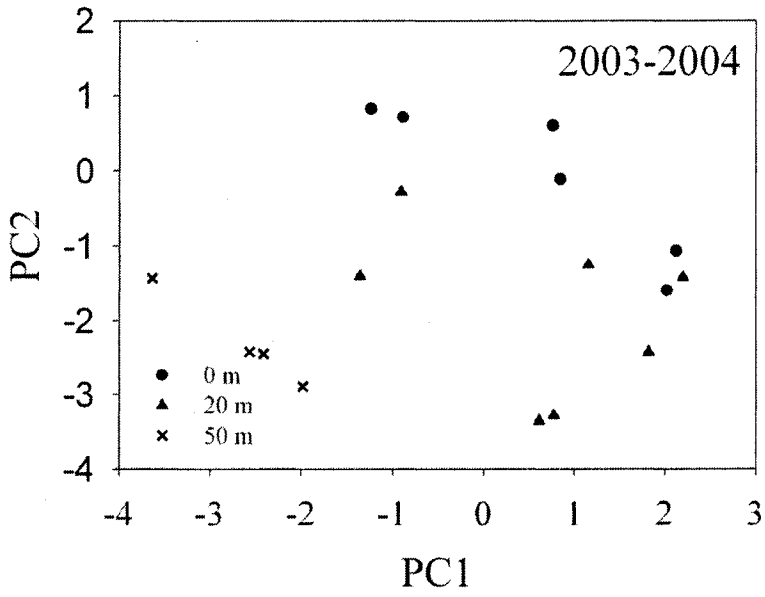
Appendix D. Principal components analysis loadings for PC2 vs. PC3.



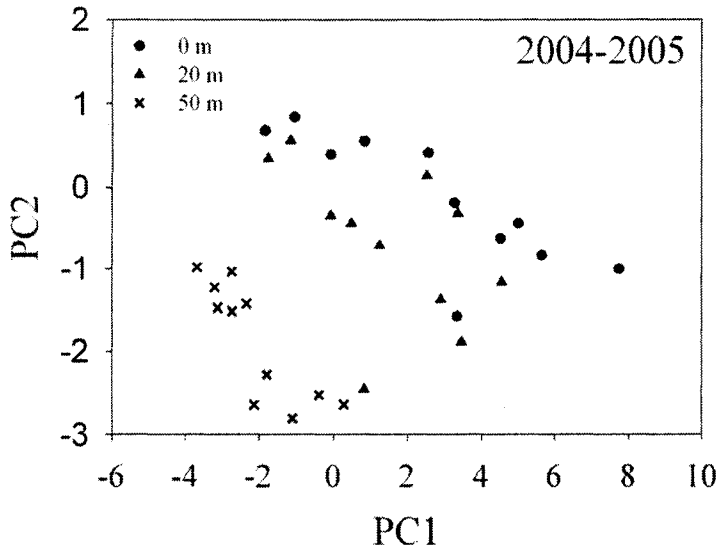
Appendix E. Principal components analysis scores plotted for PC1 vs PC2 during December 2001. (Circles, triangles, and x represent 0, 20, and 50 m, respectively).



Appendix F. Principal components analysis scores plotted for PC1 vs PC2 during December 2003. (Circles, triangles, and x represent 0, 20, and 50 m, respectively).



Appendix G. Principal components analysis scores plotted for PC1 vs PC2 during December 2004. (Circles, triangles, and x represent 0, 20, and 50 m, respectively).



Appendix H. December 2003-February 2004 Sediment trap fecal pellet morphological properties. Dashes represent that there were no pellets in that category.

Sample	Ovoid Pellets		Tabular Pellets		Spherical Pellets	Forams		Unknown Pellets		Total Pellets
	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Diameter (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	
Xiphias Day 365										
N	80				6	10		2	2	100
Mean	234.92	227.03	-	-	128.89	319.12	321.54	443.04	84.39	
Standard Deviation	153.20	122.97	-	-	130.49	71.61	39.95	0.00	0.00	
Xiphias Day 371										
N	57		3		11					71
Mean	260.97	198.51	1025.76	184.55	166.16	-	-	-	-	
Standard Deviation	119.93	85.91	405.45	88.01	97.11	-	-	-	-	
Xiphias Day 373										
N	48				10			1		59
Mean	224.08	152.73	-	-	124.13	-	-	270.64	147.68	
Standard Deviation	149.37	90.87	-	-	69.84	-	-	-	-	
Xiphias Day 389										
N	74				8	1		-	-	83
Mean	298.60	215.62	-	-	112.04	291.74	253.16	-	-	
Standard Deviation	96.97	72.29	-	-	52.34	-	-			
Xiphias Day 401										
N	72				2			4		78
Mean	331.79	189.82	-	-	209.56	-	-	388.33	124.56	
Standard Deviation	113.31	72.11	-	-	122.40	-	-	373.29	35.62	
Callinectes Day 364										
N	63		1		15			1		80
Mean	332.23	201.08	340.19	340.19	157.69	-	-	307.78	307.78	
Standard Deviation	155.62	75.80	-	-	98.11	-	-			
Callinectes Day 370										
N	44		2		30			10		86
Mean	240.53	103.43	246.47	145.87	148.67	-	-	305.91	151.73	
Standard Deviation	175.93	57.33	169.55	62.23	91.03	-	-	444.81	99.45	

Appendix H., cont. December 2003-February 2004 sediment trap fecal pellet morphological properties. Dashes represent that no pellets were observed for that category.

	Ovoid Pellets		Tabular Pellets		Spherical Pellets	Forams		Unknown Pellets		Total
Sample	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Diameter (μm)	Length (μm)	Width (μm)	Length (μm)	Width (μm)	Pellets
Callinectes Day 374										
N	53		1							54
Mean	264.57	172.52	476.62	63.29	–	–	–	–	–	
Standard Deviation	124.75	64.77	–	–	–	–	–	–	–	
Callinectes Day 388										
N	94		1		4	1		15		115
Mean	310.14	215.4	210.97	267.02	147.07	1042.3	291.74	502.27	322.84	
Standard Deviation	173.72	123.24	–	–	76.83	–	–	147.85	96.9	
Callinectes Day 400										
N	81				8			1	1	91
Mean	299.1	168.93	–	–	219.02	–	–	1137.09	170.28	
Standard Deviation	112.54	65.11	–	–	92.57	–	–	–	–	
Total pellets for seasonal sampling period:										817

Appendix I. December 2004-February 2005 sediment trap fecal pellet morphological properties. Dashes represent that there were no pellets in that category.

Sample	Ovoid Pellets Length (μm)	Ovoid Pellets Width (μm)	Tabular Pellets Length (μm)	Tabular Pellets Width (μm)	Spherical Pellets Diameter (μm)	Forams Length (μm)	Forams Width (μm)	Unknown Pellets Length (μm)	Unknown Pellets Width (μm)	Total Pellets
Xiphias Day 360										
N	7							1		8
Mean	278.3	131.1	–	–	–	–	–	485.2	107.6	
Standard Deviation	121.5	22.4	–	–	–	–	–	–	–	
Xiphias Day 368										
N	165				4	3				172
Mean	276.0	204.2	–	–	78.2	260.6	190.9	–	–	
Standard Deviation	129.9	95.7	–	–	18.6	107.6	78.5	–	–	
Xiphias Day 374										
N	87				1			26		114
Mean	195.9	182.5	–	–	119.3	–	–	127.6	154.0	
Standard Deviation	89.5	92.2	–	–		–	–	56.6	52.9	
Xiphias Day 388										
N	101		1		2			7		111
Mean	205.3	121.1	232.1	76.1	80.2	–	–	164.2	113.4	
Standard Deviation	85.4	54.7	–	–	5.9	–	–	102.9	27.0	
Xiphias Day 392										
N	53							27		80
Mean	303.1	169.4	–	–	–	–	–	410.3	114.6	
Standard Deviation	116.5	64.4	–	–	–	–	–	438.3	52.1	

Appendix I., cont. December 2004-February 2005 sediment trap fecal pellet morphological properties. Dashes represent that there were no pellets in that category.

Sample	Ovoid Pellets Length (μm)	Ovoid Pellets Width (μm)	Tabular Pellets Length (μm)	Tabular Pellets Width (μm)	Spherical Pellets Diameter (μm)	Forams Length (μm)	Forams Width (μm)	Unknown Pellets Length (μm)	Unknown Pellets Width (μm)	Total Pellets
Calinectes Day 360										
N	26		3		9	1		11		50
Mean	269.1	144.8	817.1	231.1	118.5	165.6	126.6	576.7	93.2	
Standard Deviation	128.6	57.7	892.9	304.6	58.8	–	–	429.5	36	
Callinectes Day 374										
N	63				6	8		17		94
Mean	338.6	192.1	–	–	167.2	200.4	161.1	531	93.7	
Standard Deviation	102.5	59.7	–	–	77.3	54.5	54.3	412.1	32.3	
Callinectes Day 384										
N	145		2		8	2		11		168
Mean	326.6	184.2	146.6	540.3	162.2	191.7	199.7	591.8	396.5	
Standard Deviation	88.9	51.7	25.8	97	72.6	80.1	91.4	637.3	510.9	
Callinectes Day 392										
n	158					2				160
Mean	252.8	218.7	–	–	–	246.7	248.7	–	–	
Standard Deviation	105.4	92.5	–	–	–	15.3	22.1	–	–	
Total Pellets counted for seasonal sampling period:										957

VITA

AMY REBECCA SHIELDS

Born in Kansas City, Missouri on December 21, 1977. Graduated from high school at Shawnee Mission South High School in Overland Park, Kansas in 1996. Received Bachelor's of Science in 2000 with departmental honors and highest distinction (summa cum laude) in Environmental Studies at the University of Kansas. Worked at Woods Hole Sea Education Association as assistant scientist, sailing throughout the Sargasso Sea, Caribbean, and North Atlantic on sailing school vessels. Entered the School of Marine Science at the College of William and Mary in 2001 under graduate advisor Dr. Walker O. Smith, Jr. Successfully defended her Ph.D. in May 2007.