Marine and Terrestrial Biology

Invertebrate diversity in Taylor Valley soils and sediments

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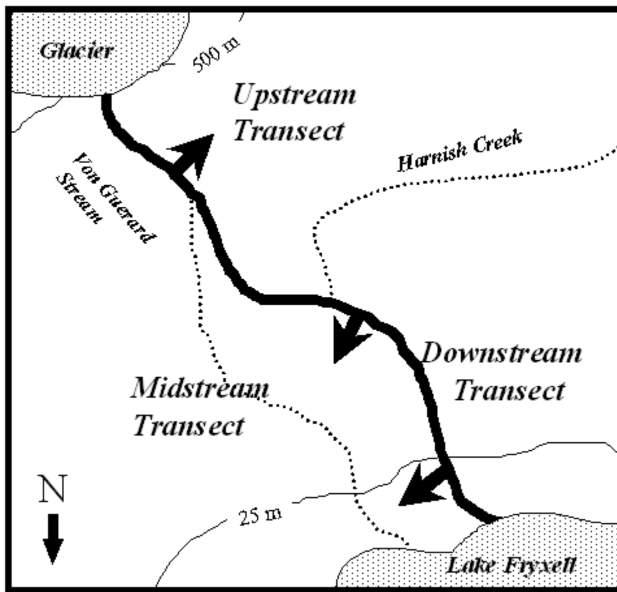
Explaining how ecosystems function across variable landscapes will require knowledge of biodiversity patterns. In particular, biodiversity studies of soils and sediments will help in understanding the linkages between ecosystem processes in both of these habitats (Freckman et al. 1997). Soils and sediments are domains for ecosystem processes such as decomposition and trace gas exchange. There are few studies, however, that have compared abundance and diversity of organisms in adjacent soils and sediments (Freckman et al. 1997). The goal of this study was to increase understanding of how the biotic communities involved in ecosystem processes are organized within an important feature of the Antarctic dry valley landscape—a stream channel and the soils and sediments surrounding it.

Dry valley streams, which flow for 6 to 10 weeks during the austral summer, are links between glaciers, lakes, and soils (Lyons et al. 1998). Glacial meltwater is carried by streams to lakes, and nutrients and salts are accumulated by the water and transported throughout the stream channel. The hyporheic zone is the area of stream sediments and adjacent soils where subsurface stream flow occurs. The hyporheic zone of dry valley streams extends out laterally, rather than deep below the stream, due to a shallow permafrost barrier. Water in the hyporheic zone accumulates salts and nutrients through weathering and atmospheric deposition. At the same time, nutrients may be lost from stream water by microbial uptake, and salts are deposited in soils and sediments when stream waters evaporate or freeze in the hyporheic zone. Inputs of nutrients and water to dry valley lakes are determined by the extent and balance of these stream and hyporheic zone interactions.

We compared invertebrate abundance and diversity in samples collected during the austral summer, 1997-1998, from the soils, benthic sediments, and hyporheic zone of the Harnish/Von Guerard Stream network (Taylor Valley, 77°S 163°E). This stream is approximately 5 km long, has an elevation change of 500 m, and empties into Lake Fryxell. Samples were collected from the top 10 cm along upstream, midstream, and downstream transects (32 m long) beginning in the sediments in the center of stream flow and extending through the channel to the soils. Nematodes, rotifers, and tardigrades were extracted and enumerated, using a sugar flotation/centrifugation technique (Freckman and Virginia 1993), and were the only invertebrates observed in these samples. Three nematode species were found (*Plectus antarcticus, Eudorylaimus antarcticus,* and *Scottnema lindsayae*).



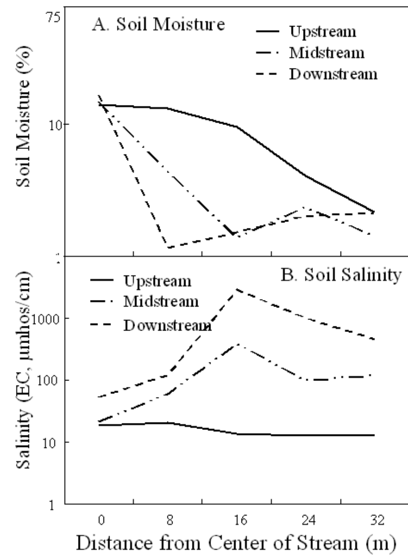


Figure 1a

Figure 1b

Figure 1a. Schematic of Von Guerard/Harnish Stream network showing the locations of the transects sampled.

Figure 1b. Moisture content (A) and salinity (B) of soil and sediment samples by location in the stream channel.

At the time of sampling, surface water was generally visible in the center of the stream channel in a 0-5 m wide stream. Moisture content declined moving out from the center to the adjacent soil system (0 to 32 m) (figure 1A). Salinity of sediments was very low in the center of the stream (0 m) (figure 1B). Samples taken 16 m from the stream center in midstream and downstream transects were the most saline. Beyond this hyporheic zone/soil transition, soils are outside the range of direct influence of stream water and associated salts and are therefore drier and less saline.

Nematodes, rotifers, and tardigrades assembled into distinct communities depending on distance from the center of stream flow and the location along the slope, upstream or downstream. Abundance and diversity of invertebrates were associated with the moisture content and salinity levels at the different locations on the stream channel. Where stream waters were flowing, at the center of the channel, the low salinity and high moisture content of the sediments were associated with communities having a high abundance of tardigrades, rotifers, and the nematode species *Plectus antarcticus* and *Eudorylaimus antarcticus* (table, figure 2).

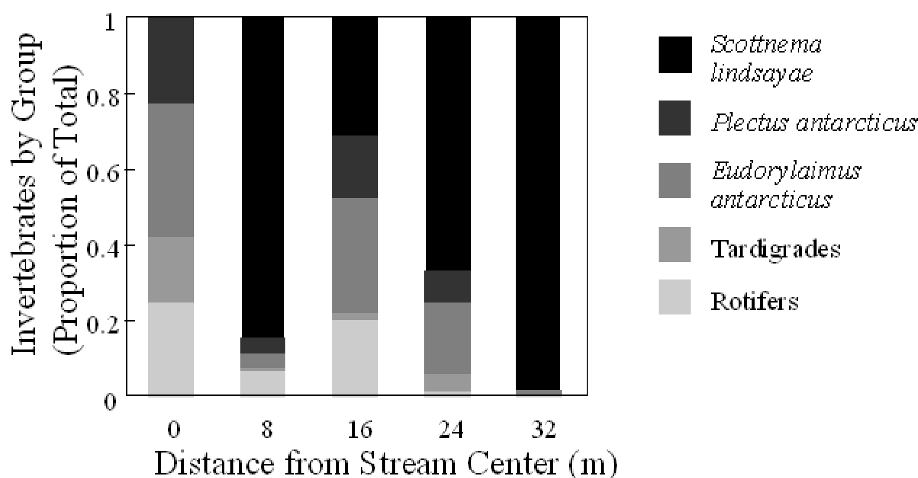


Figure 2. Invertebrate community composition along transects (all three transects are combined

Total invertebrates (nematodes, rotifers, tardigrades) per kilogram of sample at locations along transects crossing a Taylor Valley stream channel.).

Distance from Center of Stream (m)	Total # of Invertebrates
0	3320
8	1596
16	292
24	773
32	1032

The poorest habitat for invertebrates was in the hyporheic zone/soil transition (16 m), where high salinity was associated with reduced organism abundance, although communities were still diverse (table, figures 1 and 2). Outside of the stream, in the dry soils, invertebrate abundance was similar to that of the stream sediments at 0 and 8 m, but the soil communities were the least diverse (table, figure 2). Soils were dominated by a single species of nematode, *Scottnema lindsayae* (figure 2). In the downstream transect, no invertebrates were found at 16, 24, and 32 m, possibly due to excessive salinity (figure 1B).

In this study, the strong contrast between stream channel sediments and soils showed how salinity and moisture both affect the diversity and abundance of invertebrates within this feature of the dry valley landscape. Salinity affected invertebrate abundance, but not diversity. All organisms have increased density with increased moisture except the nematode *Scottnema*, which declines with increased moisture. *Scottnema* abundance is highest in the dry soils, and this organism may be adapted to specialization in the harshest (most arid) environments. Alternatively, the preferred food source for this microbivore may be more abundant in the soils. This research supports the results of previous studies, which have shown that nematodes are absent in high salinity dry valley soils (≈1,000 µmhos/cm), and there is a positive relationship

between soil moisture and abundance of *Plectus* and *Eudorylaimus* (Freckman and Virginia 1997; Powers et al. 1998).

In temperate and tropical streams, the diversity of organisms may be related to many complicated factors, including biogeography, stream characteristics, and types of local vegetation (Covich 1988). In this Antarctic stream channel, the simplicity of the ecosystem allows us to draw conclusions about how diversity compares in soils and sediments based on observations of basic habitat properties such as moisture and salinity. We have shown that invertebrates in this Antarctic stream channel are in distinct communities and environments depending on location in the stream. Studies of transitional habitats or across well-characterized environmental gradients, like the stream/soil interface of this study, coupled with measurements of ecosystem processes, should help to resolve questions about the importance of biodiversity in ecosystem functioning.

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Filtration volume and retention of carbon-14 particulate organic carbon on GF/F filters during a *Phaeocystis antarctica* bloom in the Ross Sea, Antarctica

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The spring phytoplankton blooms observed in the south-central Ross Sea polynya are dominated by the colonial haptophyte *Phaeocystis antarctica* (El-Sayed, Biggs, and Holm-Hansen 1983; Smith and Gordon 1997). A large percentage of *Phaeocystis* productivity maintains the mucilaginous substances that envelop the cells when in colonial form (Lancelot and Mathot 1985). This mucilage envelope serves to assist in predator avoidance (Verity, Villareal, and Smayda 1988), buoyancy regulation (Walsby and Reynolds 1980), and metabolic storage (Lancelot and Mathot 1985). *Phaeocystis sp.* have been reported to excrete in excess of 60 percent of their fixed carbon (Guillard and Hellebust 1971; Lancelot and Billen 1985) as dissolved organic carbon (DOC) and could represent a significant source of DOC during blooms (Lancelot and Billen 1985). Veldhuis and Admiraal (1985) cautioned, however, that disruption of colonies during filtration may introduce experimental artifact resulting in overestimation of the DOC excretion rates from *Phaeocystis* colonies. They found that when proper care was taken in isolating extracellular DOC from *Phaeocystis pouchetii*, only 14 percent of the total photosynthetic production was released as DOC.

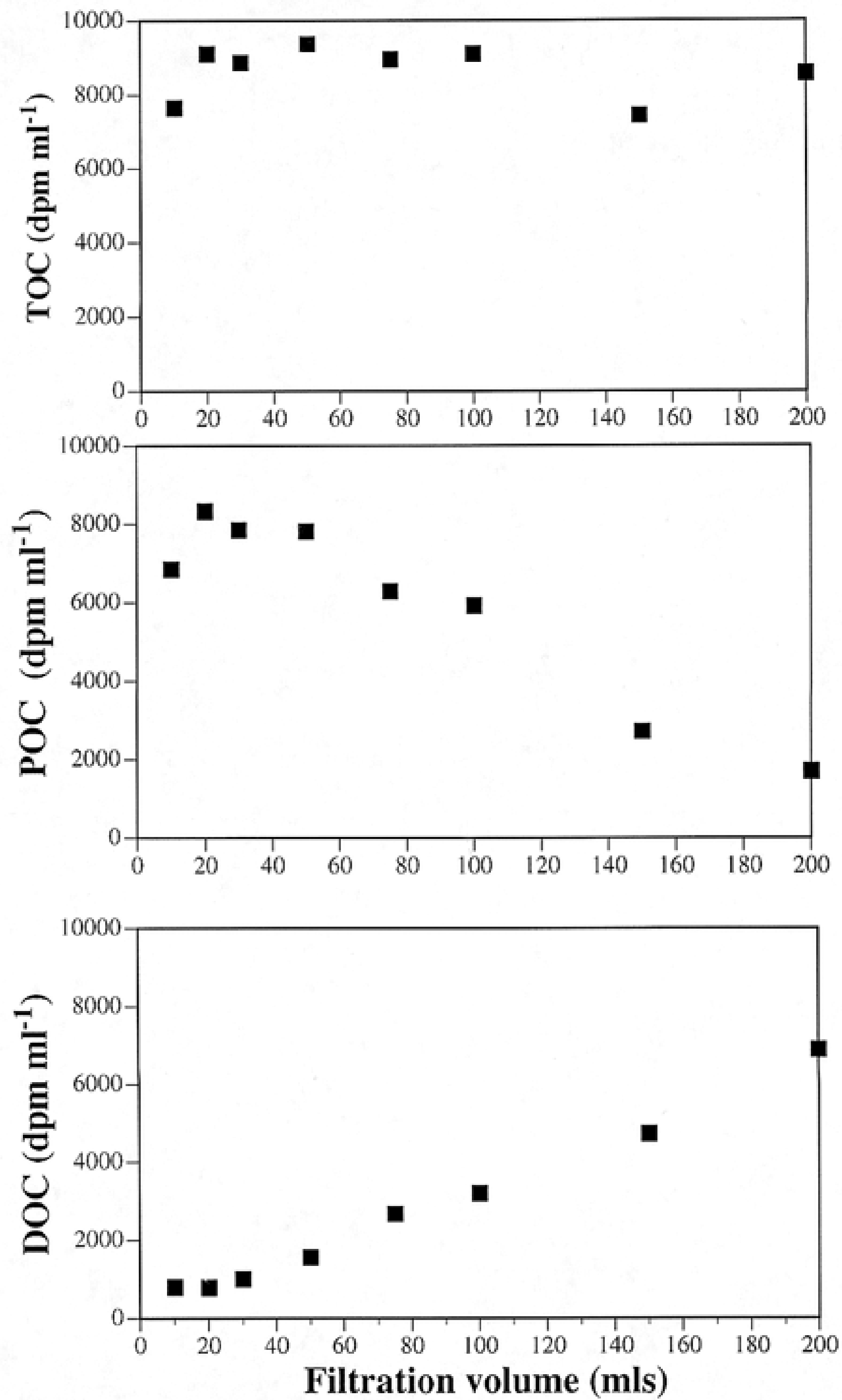
In a previous report from the Ross Sea polynya, Carlson et al. (1998) found that approximately 10 percent of the net organic carbon production during a *Phaeocystis antarctica* bloom in November and December 1994 was partitioned as DOC. In this previous study, small volumes [20–30 milliliters (ml)] of water were passed through a 47-millimeter (mm) GF/F filter to separate particulate organic carbon (POC) on the filter from DOC in the filtrate. In subsequent experimental work, however, Carlson et al. (in press) found that DOC concentrations were enhanced by 4–20 micromoles of carbon (μM C) relative to *in situ* DOC concentrations after large sample volumes (more than 5 liters) had been filtered through an 0.8 micrometer (μm) filter 142 mm in diameter. These results demonstrated the potential of introducing experimental artifact as a result of overloading POC on a filter. The question remained whether DOC release was overestimated even at smaller filtration volumes, that is, at less than 200 ml. In this study, we used freshly produced carbon-14 (^{14}C) labeled organics as a sensitive tracer to determine how filtration volumes may influence DOC release and particulate organic carbon (POC) retention on 25-mm GF/F filters. The experimental design allows insight into the effect of filtration on recently fixed carbon only, but not on the organic carbon held by the colonies for longer periods.

Samples were collected from two stations located at 169°E and 170°E along the 76°30'S transect line of the U.S. Joint Global Ocean Flux Study (JGOFS) Antarctic Environment Southern Ocean Process Study (AESOPS) in November and December 1997. Both stations represented high Phaeocystis biomass sites. Surface chlorophyll concentration levels were 9.5 and 11.8 micrograms for experiments I and II, respectively. The collection depths corresponded

to the 50 percent light level. Organic carbon was labeled with ^{14}C by following the primary production protocol of Smith and Nelson (1990). Briefly, 1 liter of surface seawater was spiked with ^{14}C sodium carbonate and incubated in deckboard incubators adjusted to *in situ* temperature and light level. Experiment I was incubated for 20 hours, and experiment II was incubated for 12 hours. Following the incubations, gentle vacuum filtration (less than 100-mm mercury) through a GF/F filter was used to partition the ^{14}C-labeled organic material into POC and DOC for sample volumes ranging from 10 to 200 ml. GF/F filters and 8 ml of each treatment's filtrate (in replicate) were acidified with 10 percent hydrogen chloride, placed on a vibrating table, and allowed to vent inorganic ^{14}C for at least 24 hours before scintillation counting.

The ^{14}C activity (in disintegrations per minute per milliliter) associated with the various organic carbon pools is presented in the figure. The ^{14}C activity of total organic carbon (TOC), the sum of POC and DOC activity, represents the amount of organic carbon produced during the incubation period. The ^{14}C activity of the TOC pool was relatively constant for all filtration treatments (figure, block *A*) allowing for further comparisons of partitioning between the POC and DOC pools as a function of filtration volume. We observed clear changes in the activity associated with the POC and DOC pools as a function of increased filtration volume. When filtration volumes exceeded 30 ml, the ^{14}C activity of the POC retained GF/F filters decreased (figure, block *B*). A concurrent increase in the ^{14}C activity of the DOC pools was also observed. If filtration volume had no effect on the partitioning of organic carbon, then one would expect the ^{14}C activity associated with respective POC or DOC pools to remain the same over the range of filtration volumes.

The fractions of labeled TOC released as DOC or retained as POC over the range of filtration volumes are shown in the table. DOC:TOC ratios were approximately 10 percent for the first 30 ml of sample filtered (table) and may be reflective of the true percentage of DOC partitioning in the Ross Sea (Carlson et al. in press). Increasing the filtration volumes to 200 ml resulted in the fraction of TOC released as DOC to as much as 80 percent and the fraction of POC retained on a GF/F filter to decrease to as little as 20 percent. These results are consistent with those of Veldhuis and Admiraal (1985) in suggesting that great care must be taken when measuring DOC release and POC retention by gelatinous *Phaeocystis* colonies. The results indicate that to minimize filtration artifact, sample volumes of 30 ml or less should be used in high biomass regions of *Phaeocystis antarctica*.



Plots of volume normalized ^{14}C activity [expressed as disintegrations per minute per milliliter (dpm ml^{-1})] associated with TOC (A), POC (B), and DOC (C) as a function of filtration volume. These panels were generated from filtration experiment II.

The percentage of TOC partitioned as DOC or POC. Values are expressed as the ratio of ^{14}C label in POC and DOC relative to ^{14}C label in TOC after simulated in situ incubation.

	Experiment I*		Experiment II**	
Volume filtered (mls)	**DOC:TOC**	**POC:TOC**	**DOC:TOC**	**POC:TOC**
10	0.06	0.94	0.11	0.89
20	0.08	0.92	0.09	0.91
30	0.16	0.84	0.12	0.88
50	0.24	0.76	0.17	0.83
75	0.25	0.75	0.30	0.70
100	0.37	0.63	0.39	0.61
150	0.49	0.51	0.63	0.37
200	0.51	0.49	0.80	0.20

*Sample incubated for 20 hours.

**Sample incubated for 12 hours.

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The chemistry of the Ross Sea in the austral spring

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In 1996 and 1997 the Joint Global Ocean Flux Study (JGOFS) supported by the National Science Foundation (NSF) conducted four process cruises in the Ross Sea. These studies were made to examine the changes and controls on the carbon cycle throughout the year. Researchers measured

- carbonate system parameters (pH; total alkalinity, TA; total inorganic carbon dioxide, TCO_2);
- nutrients-nitrate (NO_3), nitrite (NO_2), ammonia, (NH_4); phosphate, (PO_4) and silicate, SiO_2) and;
- oxygen at several locations-most were concentrated at seven stations along latitude76ºS from longitude 169ºE to 179ºW.

In this report, we briefly discuss the results from the process 1 cruise that took place in the austral spring (October 2 to November 8, 1996). The data from process 1 represent conditions at the very beginning of the "growing season".

Spectrophotometric pH (Clayton and Byrne, 1993), titration alkalinity TA (Millero, 1993), and coulometric TCO_2 (Johnson et al., 1993) were determined by established techniques (Dickson and Goyet 1994). The temperature, salinity, and oxygen were determined using JGOFS protocols (SCOR, 1996). Nutrients were determined as outlined by Gordon et al. (1993). Throughout the cruise, measurements were made on certified reference material (CRM) to monitor the accuracy and reproducibility of the CO_2 measurements. The standard deviations from the mean of pH, TA, and TCO_2 were found to be ± 0.001, ± 2 $\mu mol\ kg^{-1}$ and ± 2 $\mu mol\ kg^{-1}$, while the accuracies are approximately twice these values. The reproducibilities standard deviation from the mean of the oxygen and nutrient measurements were approximately

- ±0.5 $\mu mol\ kg^{-1}$ in NO_3,
- ±0.02 $\mu mol\ kg^{-1}$ in PO_4,
- ±1 $\mu mol\ kg^{-1}$ in SiO_2, and
- ±2 $\mu mol\ kg^{-1}$ in O_2.

The TA and TCO_2 data measured on the cruise are plotted versus salinity in figure 1. The measured values can be represented by

$$TA = 51.88 + 66.2324\,S \quad (1)$$

$$TCO_2 = -93.68 + 67.1980\,S \quad (2)$$

with standard errors of ± 2 μmol kg^{-1} in TA and ± 3 μmol kg^{-1} in TCO_2. Assuming linear, two end-member mixing of Ross Sea water with meltwater of zero salinity and ca. Zero dissolved inorganic carbon content, one can "normalize" the TA and TCO_2 data to constant salinity in order to examine the changes due to chemical and biological processes and physical processes other than simple mixing. The normalized values are defined by NTA = 35 x TA/S and $NTCO_2$ = 35 x TCO_2/S. The measurements made during the cruise give NTA = 2371 ± 2 μmol kg^{-1} and $NTCO_2$ = 2258 ± 3 μmol kg^{-1}. These values can be compared with the measurements recently made by Bates et al. (1998) of NTA = 2387 ± 8, 2377 ± 6 μmol kg^{-1} and $NTCO_2$ = 2186 ± 27, 2228 ± 24 μmol kg^{-1}, respectively, in November and December 1995 in the Ross Sea. The values of NTA are higher than our values by 7 to 17 μmol kg^{-1} while the values of $NTCO_2$ are lower. The higher values of NTA are related to the loss of NO_3 due to diatom blooms.

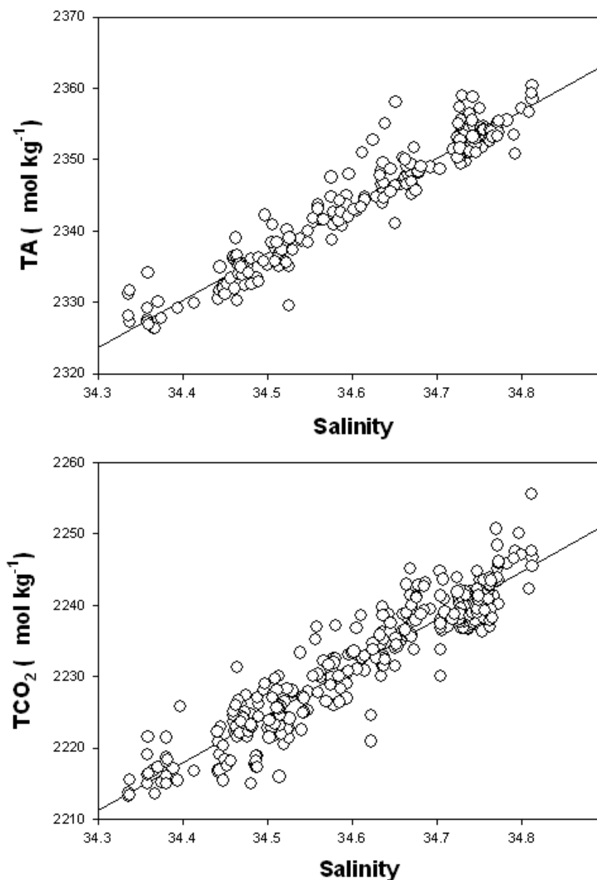

Figure 1. The total alkalinity (TA) and total carbon dioxide (TCO_2) in the Ross Sea as a function of salinity.

Profiles of all the physical and chemical properties measured during the cruise (with data more than 300 depending on the property) are shown in figures 2 to 3 and summarized in the table.

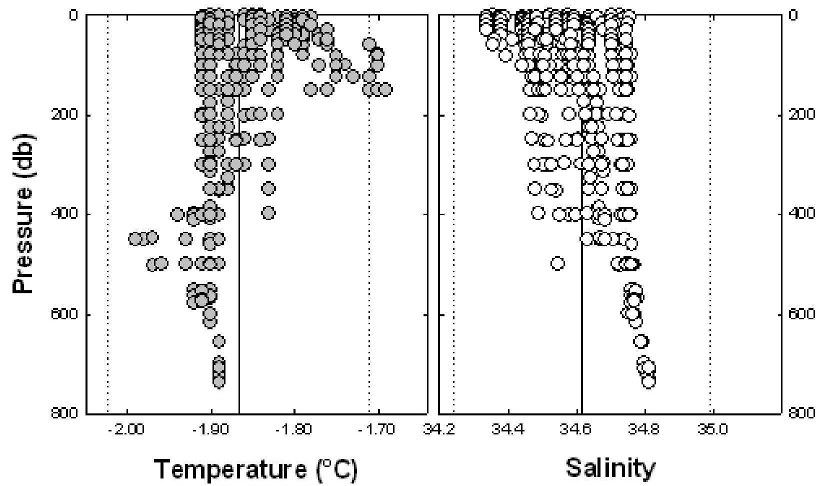

Figure 2a. The temperature and salinity in the Ross Sea as a function of depth. The solid vertical line represents the mean value. (db denotes decibar)

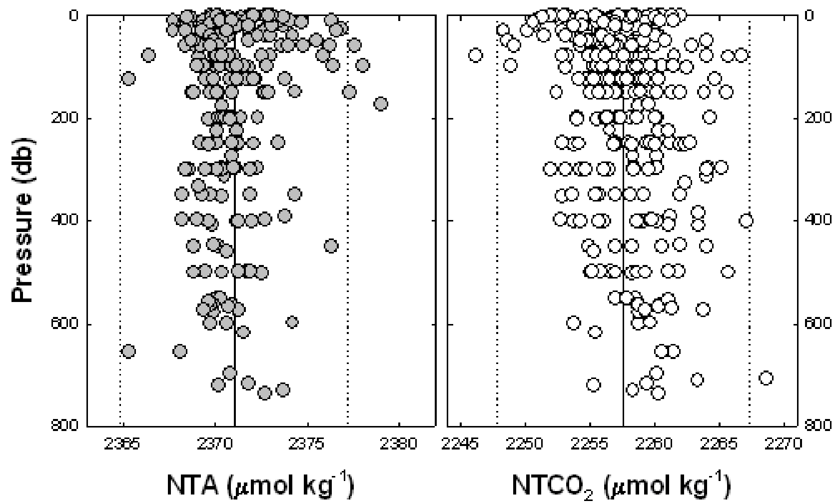

Figure 2b. The normalized total alkalinity (NTA) and total carbon dioxide ($NTCO_2$) in the Ross Sea as a function of depth. The solid vertical line represents the mean value. (db denotes decibar).

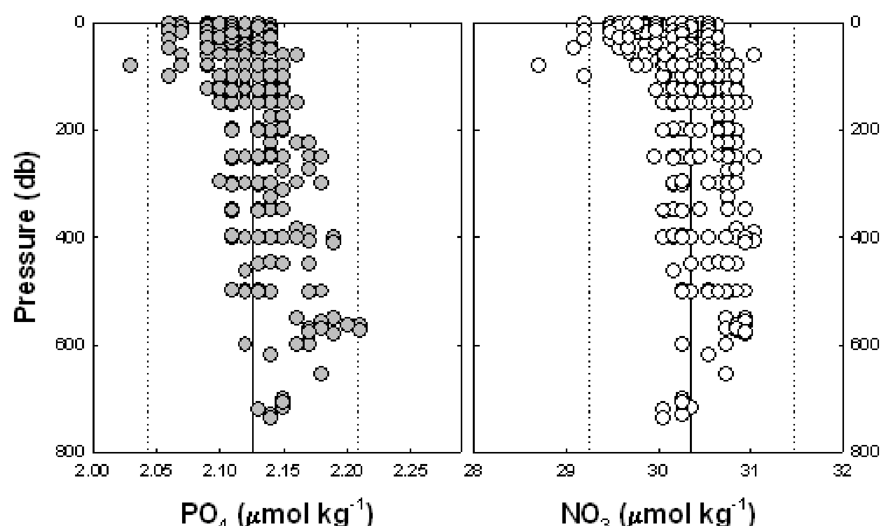

Figure 2c. The nitrate and phosphate in the Ross Sea as a function of depth. The solid vertical line represents the mean value. (db denotes decibar).

A summary of the average physical and chemical properties of the Ross Sea waters in the austral spring (the errors represent 1 σ).

Property	**Mean Value**	**Property**	**Mean Value**
Temperature (^oC)	-1.86 ± 0.06	Salinity	34.6 ± 0.1
Oxygen (μmol kg^{-1})	284 ± 5	Nitrate (μmol kg^{-1})	30.3 ± 0.3
Phosphate (μmol kg^{-1})	2.13 ± 0.03	Silicate (μmol kg^{-1})	76 ± 2
pH(spec), 25^oC	7.606 ± 0.006	TA (μmol kg^{-1})	2345 ± 9
NTA (μmol kg^{-1})	2371 ± 2	TCO_2 (μmol kg^{-1})	2232 ± 9
$NTCO_2$ (μmol kg^{-1})	2258 ± 3	pH (*in situ*), calc	8.03 ± 0.03
pCO_2 (*in situ*), calc	398 ± 33		

As is apparent from these results, the waters sampled during the process 1 study tend to be uniform throughout the water column in the austral spring. This is not surprising because low ambient air temperatures and details of the salinity structure suggest active ice-formation and convection during this season. The temperature and salinity as a function of depth shown in figure 2a yield average values and standard deviations of $t = -1.86 \pm 0.06^oC$ and $S = 34.6 \pm 0.1$. Profiles taken near the Ross Ice Shelf revealed the presence of supercooled (relative to 1 atm) waters arising from subsurface contact of ambient water with the Ross Ice Shelf (temperature values of approximately -2 in figure 2a). The scatter in values of T and S arises from freezing and intrusions of relatively warm Modified Circumpolar Deep Water (MCDW).

The values of NTA, $NTCO_2$, NO_3, PO_4, O_2 and SiO_2 shown in figures 2b, 2c, and 3a tend to be quite constant over the entire water column. The chemical properties are not strongly affected by the physical processes affecting T and S. The SiO_2, however, is slightly higher in the MCDW and may be affected by dissolution from the sediments in the near bottom waters. The calculated *in situ* pH and pCO_2, using the constants of Mehrbach et al. (1973) and the program of Lewis and Wallace (1998) are shown in figure 3b. The average values are quite reasonable (pH = 8.03 ± 0.03 and pCO_2 = 398 ± 33 μatm). The apparent depth dependence of pH is related to the effect of pressure on the thermodynamic constants. The measurements of the pH as a function of depth determined at 1 atm are constant over the entire depth range (pH = 7.606 ± 0.006). The mean values of the chemical properties given in the table can be used to examine the changes that occur in the Ross Sea throughout the study due to biological oxidation and production and water mass mixing. The N/P ratio (14.2 ± 0.4) and Si/N ratio (2.5 ± 0.1) are in reasonable agreement with the recent measurements of Bates et al. (1998) (N/P = 14 and Si/N = 3.0).

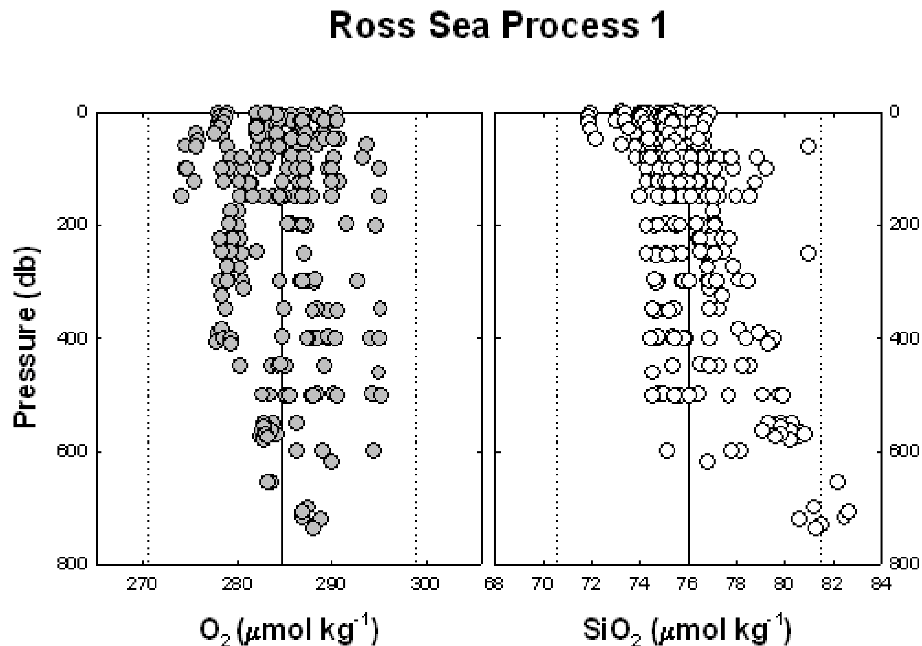

Figure 3a. The oxygen and silicate in the Ross Sea as a function of depth. The solid vertical line represents the mean value. (db denotes decibar).

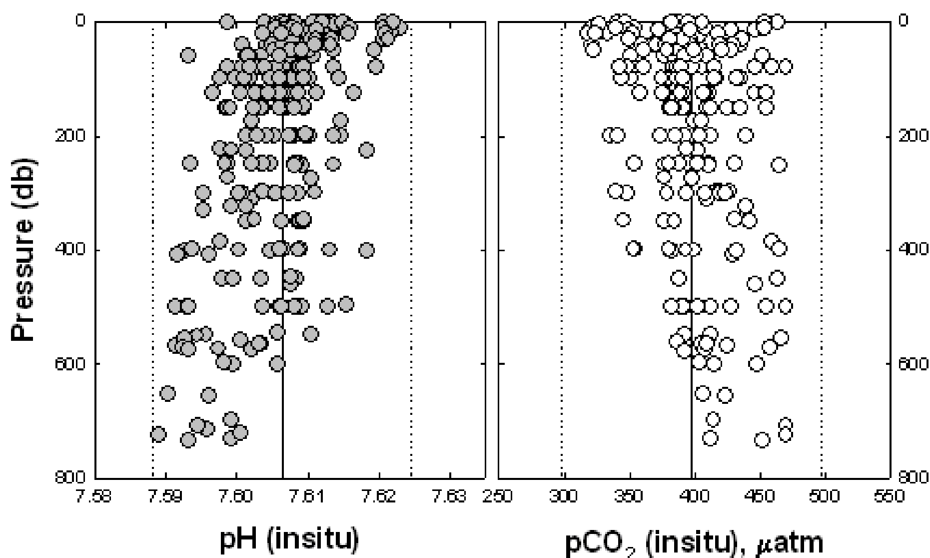

Figure 3b. The pH and pCO_2 in the Ross Sea as a function of depth. The solid vertical line represents the mean value. (db denotes decibar).

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Bio-optical measurements and modeling within the Southern Ocean

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Models describing the biogeochemical cycling of carbon in the Southern Ocean require information on the time and space resolution of planktonic biomass, as well as rates of primary production. Optical measurements of seawater provide a useful proxy for these reservoirs and processes. When coupled with the use of remote-sensing platforms (e.g. moorings, satellites) these optical measurements can greatly enhance the observational value of traditional shipboard studies. Bio-optical models used to describe the coupling between the planktonic community and seawater optical properties have been developed and parameterized primarily from measurements in lower latitude waters; however, evidence from previous studies suggests that these relationships can differ significantly in polar oceans (for example, Mitchell and Holm-Hansen 1991). The goals of our project are to develop improved bio-optical algorithms for the Southern Ocean, and to examine the underlying mechanisms that contribute to regional differentiation.

The emergence of new instruments for ocean color remote sensing, such as the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) launched in August 1997 by the National Aeronautics and Space Administration (NASA), has provided further impetus for improving our understanding and interpretation of satellite imagery. The basis of ocean color remote sensing is conceptually simple. A fraction of the solar radiation that enters the ocean is backscattered within the upper surface layers, and can be detected above the ocean surface by an appropriate sensor. The intensity and spectral composition of this upwelling light is largely determined by the absorbing and scattering properties of seawater, which in turn are strongly influenced by the particles comprising the planktonic community. Variability in ocean color thus contains information on biological processes occurring in the upper surface layer of the ocean.

During the 1997-1998 field season, we participated in three cruises as part of the Antarctic Environment and Southern Ocean Process Study sponsored by the U.S. Joint Global Ocean Flux Study. One cruise was within the Ross Sea (November-December 1997), followed by two subsequent cruises within the Antarctic Polar Front Zone along 170º W (January-March 1998). On these expeditions, we completed nearly 100 continuous *in situ* profiles of spectral reflectance, absorption, beam attenuation, and backscattering to a depth of 200 meters with an integrated optical profiling system. In parallel with these measurements, discrete water samples were collected from the ship's CTD-Rosette for laboratory analyses of pigment composition and concentrations, particulate organic carbon concentration, particulate and dissolved spectral absorption, particle number and size distribution.

During the Ross Sea Process IV cruise, high-resolution SeaWiFS data were acquired by ASA staff at McMurdo station. This data was transferred to the Arctic and Antarctic Research Center at Scripps Institution of Oceanography for cataloging and archiving, and processed in our

laboratory using a modified version of the SeaDAS software (version 3.2) available from the NASA SeaWiFS project. Figure 1 is a one-week composite of images illustrating surface chlorophyll-*a* concentrations in the Ross Sea calculated using the Mitchell's antarctic algorithm (Mitchell 1992). Although a large portion of the image is masked by cloud cover, considerable mesoscale variability of surface chlorophyll-*a* concentration is apparent. The elevated surface chlorophyll-*a* concentration in the western portion of the study region (near station A) result from a bloom of the Prymnesiophyte *Phaeocystis antarctica*.

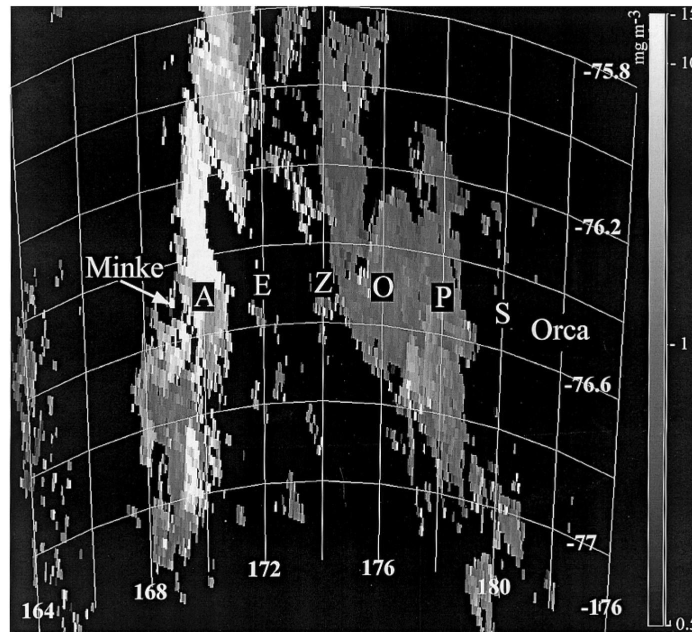

Figure 1. Grey-scale SeaWiFS image illustrating a one-week composite of surface chlorophyll-a concentrations in the Ross Sea from 26 November to 3 December 1997.

Despite persistent cloud cover, we were able to make some direct comparisons between ship-based measurements and SeaWiFS data collected within several hours of each other. These comparisons indicate that the normalized water-leaving radiances retrieved by the satellite were consistent with our surface measurements. For low-to-moderate surface chlorophyll-*a* concentrations, a preliminary analysis comparing two different algorithms suggests that the Mitchell (1992) algorithm provides better pigment retrieval than the standard NASA Ocean Color 2 algorithm. In regions with high-surface chlorophyll-*a*, such as the western region of the Ross Sea, both algorithms appear to significantly overestimate actual pigment concentrations.

Our comprehensive suite of *in situ* measurements will ultimately allow us to examine the underlying causes of such variations, and to develop improved bio-optical algorithms. The spectral reflectance is largely a function of two seawater optical properties, the spectral coefficients for absorption and backscattering. Although the absorption coefficients of oceanic waters have been extensively studied over the past decade, a lack of appropriate instrumentation for measuring backscattering has resulted in few observational studies. A unique feature of our profiling system was the inclusion of a new submersible instrument (Hydroscat-6, HOBI Labs)

designed to measure the backscattering coefficient in six spectral bands (Maffione and Dana 1997).

Figure 2 contrasts two stations occupied during a rapid east-west transect in the Ross Sea during the first half of December, 1997. Station 431 [76° 31' S 179° 56' E], near the eastern end of the transect and labeled Station S in Figure 1, was characterized by low surface concentrations of Chl (~1.5 mg m^{-3}) and particulate organic carbon (POC). Station 437 [76° 29' S 168° 56' E], located at the western end of the transect (Minke) within the *Phaeocystis* bloom, had significantly elevated concentrations of chl-*a* and POC in the surface layer. The panels on the right illustrate vertical profiles of the backscattering coefficient for three different spectral bands at these stations, and these profiles qualitatively match vertical distributions of chl-*a* and POC. Measured backscattering coefficients in the surface layer are three to four times, depending on light wavelength, higher in the bloom station and there are significant differences in the spectral shape. However, backscattering per unit pigment is approximately 30% lower in the surface of the bloom station (Station 437) as compared to Station 431.

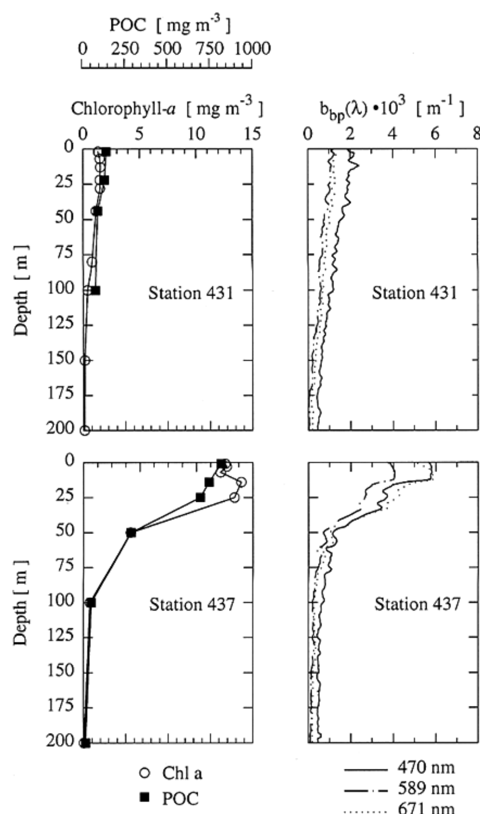

***Figure 2.** Vertical distributions of the concentration of chlorophyll-a and particulate organic carbon (POC) for Station 431 (upper left panel) and Station 437 (lower left panel). For each station, the panel to the right depicts concomitant measurements of the particulate backscattering coefficient at three distinct light wavelengths. Both stations were occupied during the 1997 AESOPS Process IV cruise in the Ross Sea.*

Figure 3A compares surface particulate absorption spectra, normalized to chlorophyll-*a* concentration, for these two stations. Throughout the visible spectrum, we observe only small differences between the two stations. Both spectra exhibit a pronounced absorption peak in the

ultraviolet region around 330 nanometers, which we hypothesize indicates the presence of high concentrations of mycrosporine-like amino acids. These compounds are thought to play a photoprotective role in preventing UV damage in phytoplankton cells. Consistent with this hypothesis, a decrease in the relative magnitude of this absorption peak with increasing depth in the water column is observed at Station 437 (Figure 3B).

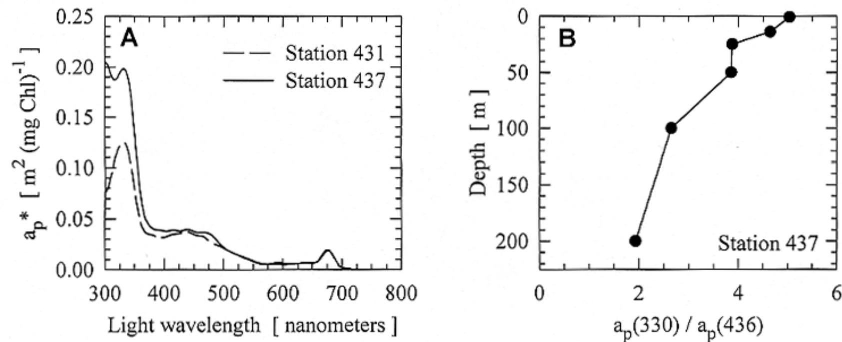

Figure 3. ***(A) Surface values of the particulate absorption coefficient, normalized to chlorophyll-a concentration, as a function of light wavelength at two stations within the Ross Sea. (B) Depth variation in the ratio of particulate absorption at two light wavelengths (330 nm to 436 nm) for Station 437 in the Ross Sea.***

Our preliminary analyses offer further evidence that ocean color algorithms for the Southern Ocean differ from temperate ocean models. Mitchell (1992) proposed that variability in pigment-specific absorption coefficients were an important component contributing to this deviation. Our results suggest that variability in pigment-specific backscattering may also be an important contributor to variability in reflectance algorithms. We are currently investigating how these changes are related to variability in the particulate assemblage, including planktonic species assemblage.

This research was supported by the National Aeronautics and Space Administration contract NAG5-7100 and the National Science Foundation grant OPP 98-02836. We thank Berzas Bichnevicius for assistance in the collection of field data. We also thank Jon Alberts, Janet Barnes, Antarctic Support Associates, and the officers and crew of the R/V *Nathaniel B. Palmer* and the R/V *Roger Revelle* for outstanding logistical support.

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Distribution of invertebrate larvae in relation to physical structure and UVB-light intensity in the water column off Anvers Island, Antarctic Peninsula

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For 20 years, springtime declines in stratospheric ozone over Antarctica have led to increases in intensities of ultraviolet-B radiation [UV-B; wavelengths of 280-320 nanometers (nm)] that penetrate the surface waters of the ocean and can cause significant damage to biological systems. These springtime increases represent a potential hazard to the survival of antarctic marine organisms (Karentz 1991).

The antarctic coastal zone is inhabited by a diverse and abundant fauna of benthic (bottom-dwelling) invertebrates. Benthic adults are largely shielded from UV-B damage by the overlying water column. Many also possess morphological, biochemical, and behavioral defenses, including shells and UV-absorbing compounds. Many antarctic invertebrates reproduce by releasing gametes freely into the water (Pearse, McClintock and Bosch 1991). Their embryonic and larval life stages are spent adrift in the water column. Embryos and larvae that are dispersed into surface waters may be exposed to potentially damaging levels of UV-B light, particularly during periods of ozone depletion. During the spring and summer in 1996-1997 and 1997-1998 at Palmer Station (64° 46' S, 64° 04' W), our group studied the effects of UV-B on the eggs, embryos, and larvae of benthic marine invertebrates. One primary objective of this work was to determine the vertical and seasonal distribution of larvae, relative to the penetration of UV-B and to the physical structure (density, salinity, temperature) of the water column. Initial results from this component of our study are presented here.

We collected plankton by towing a net [30-centimeters mouth diameter and 153-micrometer (µm) mesh] from a small boat at several stations, primarily over deep waters (40-100 meters) just outside of Arthur Harbor on the southeast coast of Anvers Island. Each net was equipped with a digital flowmeter and a close-open-close system (General Oceanics CC5100 series), which allowed us to collect quantitative samples from discrete depths. Intensities of UV-B (305 nm, 320 nm), UV-A (340 nm, 380 nm), and photosynthetically active radiation (400-700 nm) from the surface down to 20-50 meters depth were measured with a profiling radiometer (Biospherical Instruments Model PUV500) . We also determined the temperature, salinity, and density of the upper water column (to a depth of 50 meters) with a Seacat Profiler SBE-19 CTD system.

We sampled approximately 6,800 cubic meters (m^3) of water between September 1997 and February 1998. The average sample volume per tow was 50 m^3. Overall, larval diversity and larval numbers were relatively low, compared to collections made at the more northerly Signy Island (Stanwell-Smith, Clark, Perry and Todd 1999). The number of larvae collected per 10 m^3

of water was 5.2 plus-or-minus 10 (mean ± standard deviation), and the highest abundance was 19.2 larvae per 10 m^3. The most common larvae were sea star larvae [assignable to the genus *Odontaster* (figure 1A)], several types of larvae of nemertean worms [belonging primarily to the ubiquitous species *Parborlasia corrugatus* (Peck, 1993)], and tunicate larvae (representing two or more unidentified forms). Several types of snail larvae were collected, including one that we assigned to the common limpet, *Nacella concinna* (figure 1B). Representative abundance profiles are shown in figure 2. In the 19 November collection, we collected sea star and nemertean larvae at depths ranging from 4-17 m (figure 2A). Combined densities at 4 m were relatively high (6.5 per 10 m^3). Profiles taken on 30 December (figure 2B). A set of 4 diel collections made on 26 and 27 January (figure 2C) yielded only 4 larvae above a depth of 7 m, but there was no apparent diel movement of larvae toward the surface (figure 2C).

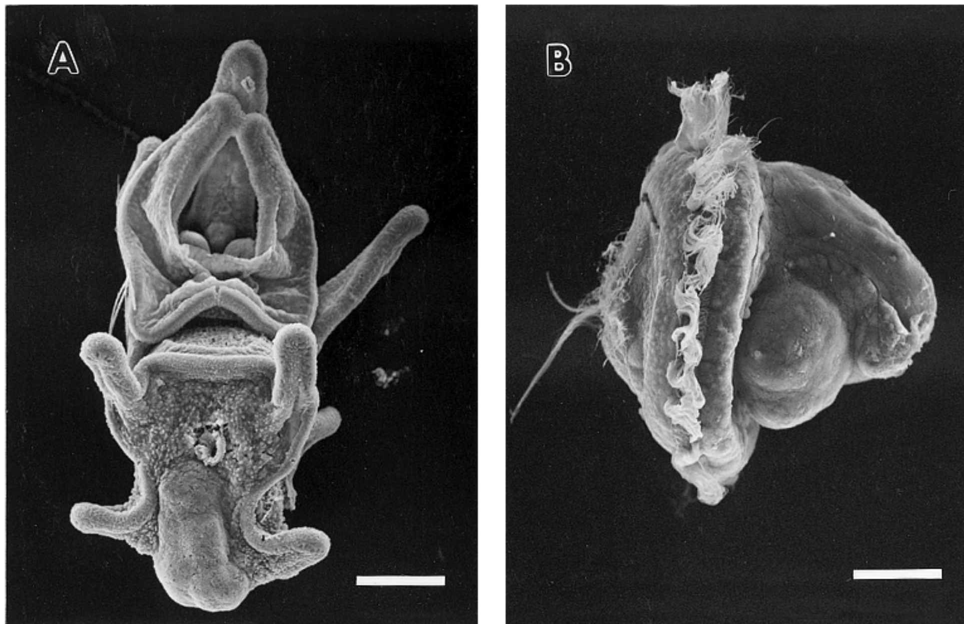

Figure 1. A. Brachiolaria larva assigned to the sea star genus **Odontaster*****. B. Veliger larva assigned to the common limpet*** **Nacella concinna. *The scales are 100 µm in (A) and 50 µm in (B).***

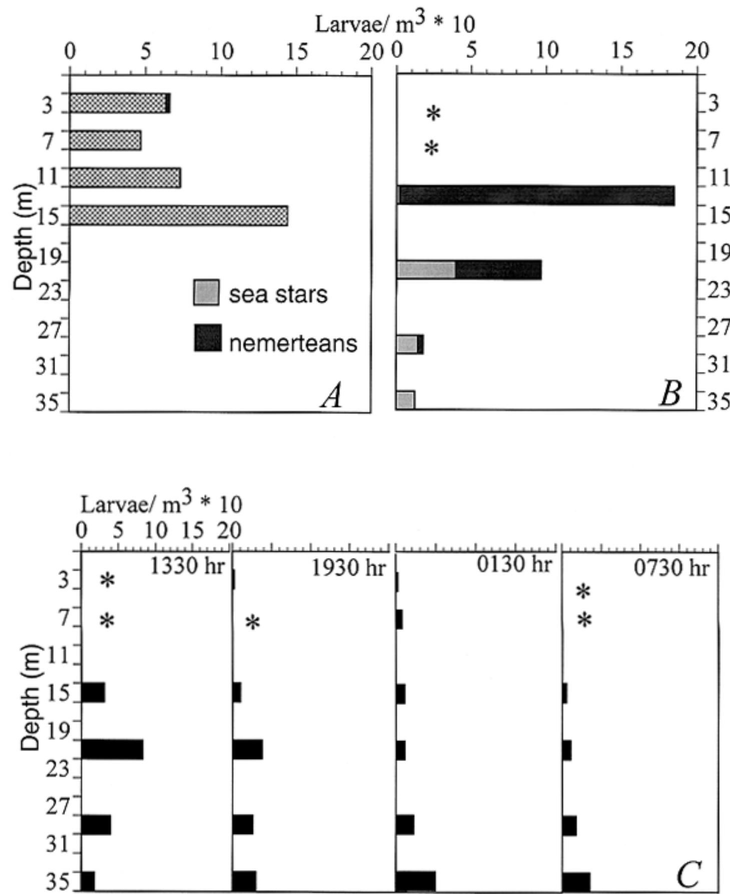

Figure 2. Sea star and nemertean larvae collected on 19 November (A) and 30 December (B). C. Numbers of all larvae (primarily sea stars and nemerteans) collected in 4 profiles taken on 26-27 January near the Joubin Islands. Asterisks indicate depths where samples yielded no larvae.

Different patterns in the surface distribution of larvae on the three sampling dates may be explained by changes in the physical structure of the water column (figure 3A). On 19 November there was no major density discontinuity layer below 3 m, and no apparent barrier to vertical mixing of the water column. On 30 December, a density gradient of about 0.2 kg per m^3 between depths of 5-10 may have limited the movement of water to the surface. No larvae were collected at the 7 m and 3 m sampling depths on this date (figure 2B). By the 26 and 27 January, warming sea-surface temperatures had produced a gradient of more than 0.8° C between 7 and 12 m depth. This temperature decrease, in conjunction with a slight increase in salinity, contributed to an increase in water density of 0.11 kg per m^3 and may explain the near absence of larvae from diel samples at 3 and 7 m depths.

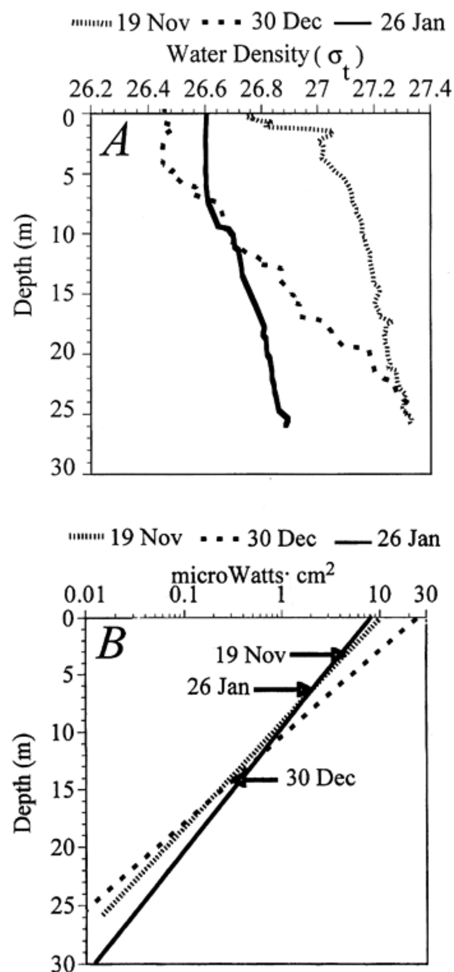

Figure 3. A. Vertical profiles of water density showing major changes with depth at 1-3 m, 5-8 m and 7-10 m for November, December, and January sampling dates. B. Best fit lines for UVB penetration into the water column for those same dates. Arrows indicate the shallowest depth at which significant numbers of larvae were collected.

Exposure of larvae to UV-B is a function of a number of variables, including ozone levels, cloud and sea-ice cover, water clarity, and depth of habitation. The UV-B levels found in surface waters during the early afternoons of our three sampling dates were highest on 30 December (figure 3B). However, on 19 November, larvae occupied a higher position in the water column and were exposed to the highest UV-B intensities (about 3.6 microWatts per cm^2). Exposures to similar intensities of UV-B for more than a few hours are known to be harmful or lethal to experimental embryos and larvae of sea stars and sea urchins (unpublished data collected by the authors). Note that the average stratospheric ozone concentration over Palmer Station on 19 November was 342 Dobson units, nearly 2.5 times higher than the lowest levels recorded during the spring of 1997. Exposures to UV-B may therefore be higher than those reported.

Initial analysis of data from this study indicates that planktonic larvae of antarctic benthic invertebrates disperse into the upper meters of the water column and are exposed to high intensities of UV-B radiation during periods of mixing in surface waters. The same physical processes that deliver planktonic embryos and larvae to surface waters may also transport them rapidly to greater depths, limiting the time they spend in the potentially hazardous upper layer of the water column.

We wish to thank R. Heine, D. Krakowski, M. Pineida, G. Wardle and ASA support personnel at Palmer Station for assistance with this research. Supported by National Science Foundation Office of Polar Program grants to I. Bosch (OPP 95-280890) and D. Karentz (OPP 95-2841).

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Bacteria-algae associations in the sea ice and upper water column of the Ross Sea in the late austral summer

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The ecological role of heterotrophic bacteria in the microbial food web of the Southern Ocean is unresolved. A coupling between phytoplankton and bacterial production is well documented in mid-to low-latitude oceans (e.g., Bird and Kalff 1984; Cole, Findlay and Pace1988) and is thought to result from the heterotrophic uptake of dissolved organic carbon (DOC) released by the primary producers (i.e. the "microbial loop;" Pomeroy 1974; Bjørnsen 1988). In Antarctic waters, however, the extent to which bacteria rely on phytoplankton production, and consequently contribute to total ecosystem production, is disputed. A positive correlation between algal and bacterial biomass has been observed in regions of the Southern Ocean (e.g., Cota *et al*. 1990; Lochte *et al*. 1997). Conversely, variability in the strength of this correlation, and even an uncoupling of algae and bacteria, has also been documented (e.g. Cota *et al*. 1990; Bird and Karl 1999). Accurate characterization of the microbial loop in the Southern Ocean requires quantification of algal and bacterial biomass and activity over a seasonal time scale and in the diversity of marine habitats that surround Antarctica. This necessitates that bacteria-algae associations in the pelagic environment should not be studied apart from similar associations in the sea ice that is a prominent feature of most antarctic waters.

Between 1 and 31 January 1999, we collected 125 bacteria samples from 33 sites along three longitudinal transects (135W, 150W, 165W) made by the U.S. Antarctic Program research ship *Nathaniel B. Palmer* in the eastern Ross Sea (figure 1). We primarily sampled three distinct habitats—the consolidated sea ice, the layers of slush (snow + seawater) overlying the consolidated sea ice, and the top of the water column. Core samples were collected with a SIPRE or Kovacs core barrel (inner diameter of 7 cm), sectioned, placed in 0.2 µm filtered seawater at a ratio of 3:1 seawater to ice-volume, and melted for 8-12 hours at 0-4°C. Bulk slush samples were collected using acid-washed Nalgene jars and melted at 0-4°C for 4-6 hours; slush interstitial waters were collected with sterile syringes. Water-column samples were collected with 10L Niskin bottles deployed on a Rossette (equipped with a CTD, a Fluorometer, and a PAR sensor). Samples were fixed aboard ship with phosphate-buffered glutaraldehyde (GTA) at a final concentration of 0.5%. Sub-samples ranging in volume from 0.5-60 mls were then stained with 4',6-diamidino-2-phenylindole (DAPI), filtered through 0.2 µm black polycarbonate membrane filters, and mounted onto glass microscope slides. Slides were frozen until processing at home institutions.

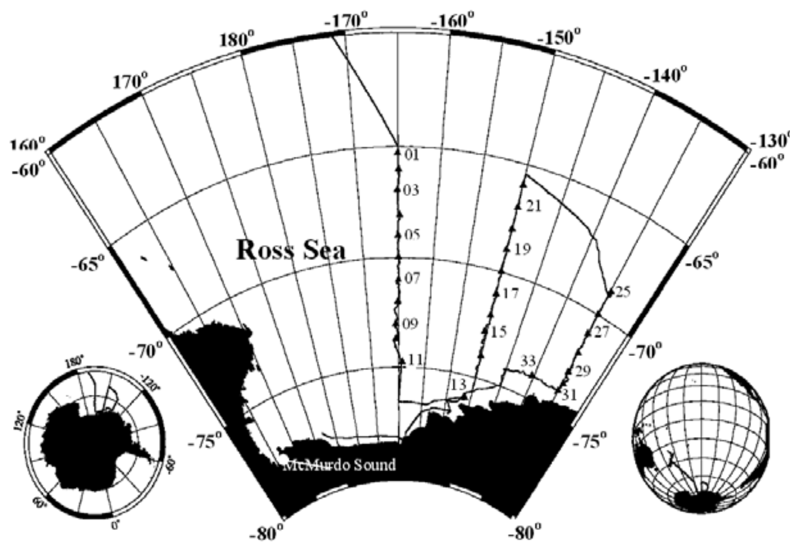

Figure 1. Track of the icebreaking research ship* Nathaniel B. Palmer *showing the 33 sites in the eastern Ross Sea at which sea ice and water column algae and bacteria were collected in January 1999.

Non-filamentous and filamentous bacteria were enumerated using epifluorescence direct count techniques (Kepner and Pratt 1994). Direct counts of bacteria from each sample were used to determine bacteria cell concentration (cells m^{-3}). Digital images were taken during counting using the imaging program Image Pro Plus and were used to size non-filamentous and filamentous cells. Total bacterial biomass (non-filamentous and filamentous bacteria; mg C m^{-3}) was determined using cell concentration values, cell-size estimates, and a conversion factor for bacterial carbon per volume that is based on data over a range of cell sizes (Simon and Azam 1989). Chlorophyll-*a* concentrations ($\mu g\ l^{-1}$) were determined fluorometrically. Linear regressions of log-transformed data sets were used to test for a correlation between algal biomass (expressed as chl *a* concentration) and bacterial biomass.

In the Ross Sea, the mean total bacterial biomass in the upper water column was significantly lower than that in the consolidated sea ice and slush—1.68 mg C m^{-3} in the water column versus 19.17 and 24.13 mg C m^{-3} in the sea ice and slush, respectively ($p<0.01$, Student's t-test). Bacteria cell concentration and total bacterial biomass corresponded positively and significantly with algal biomass in the sea ice and slush ($p<0.05$ for least squares regressions; table). The inverse was true at the surface of the water column below the ice where algal biomass was negatively correlated with both bacteria cell concentration and total bacterial biomass ($p<0.02$ and 0.13, respectively, for least squares regressions; table). However, analysis of the combined data for the water column, sea ice, and slush revealed a significant positive relationship between algal and bacterial biomass across the range of sample types ($P<0.00001$ for least squares regression; table; figure 2).

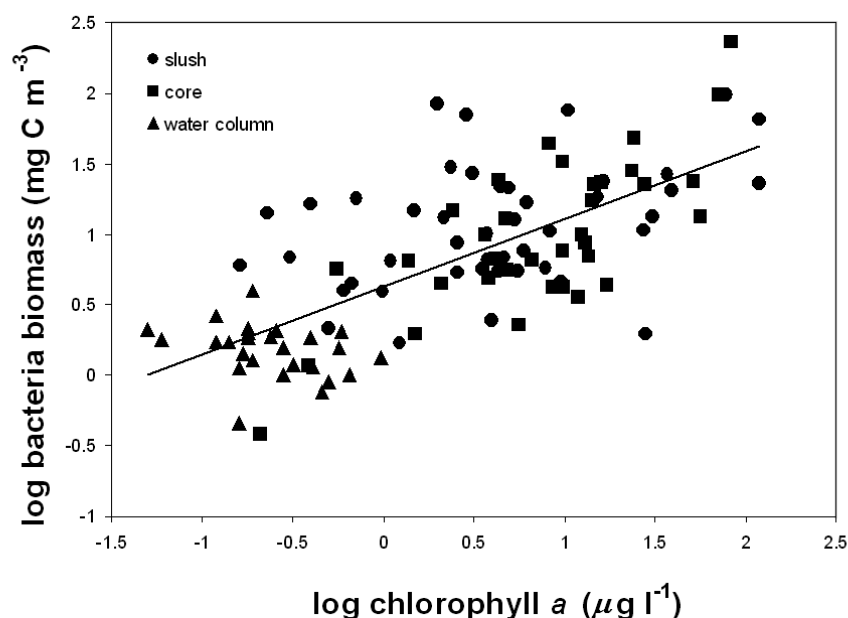

Figure 2. Results of a least squares linear regression of log-transformed total bacterial biomass and chlorophyll a *concentration [chl* a*] amongst habitats sampled in the Ross Sea. The linear regression equation is log [biomass] = 0.4805 (log[chl* a*]) + 0.6298, r^2 = 0.5051*

Regression statistics for log_{10} transformed data for bacterial biomass (BB, mg C m^{-3}) vs. chlorophyll* a *(CHLA, $\mu g\ l^{-1}$) and bacterial cell concentration (BC, cells m^{-3}) vs. CHLA for slush, ice core, water column, and combined (slush, core, and water column) data sets. Symmetrical 95% confidence limits (CL) are given for geometric mean (GM) and least squares (LS) slopes. P-values (p), y-intercepts (intercept), and R^2 values (r^2) are for LS regressions.

Habitat	y, x	N	GM slope(CL)	LS slope CL)	p	intercept	r^2
slush	BB, CHLA	45	0.6251 (0.18)	0.24 (0.18)	0.009	0.91	0.147
	BC, CHLA	45	0.5855 (0.17)	0.19 (0.17)	0.030	11.47	0.104
ice core	BB, CHLA	35	0.8948 (0.21)	0.66 (0.21)	0.00001	0.41	0.554
	BC, CHLA	35	0.9149 (0.20)	0.72 (0.20)	0.00001	11.00	0.620
water column	BB, CHLA	26	-0.6122 (0.25)	-0.19 (0.25)	0.127	0.06	0.094
	BC, CHLA	26	-0.6311 (0.24)	-0.29 (0.24)	0.016	10.97	0.218
combined	BB, CHLA	106	0.6761 (0.09)	0.48 (0.04)	0.00001	0.63	0.505
	BC, CHLA	106	0.5554 (0.09)	0.33 (0.04)	0.00001	11.36	0.357

The lack of correlations between algae and bacteria in the water column of the eastern Ross Sea suggests an uncoupling similar to that observed by Bird and Karl (1999) during springtime phytoplankton blooms in the Gerlache Straight. The authors attribute suppression of bacterial biomass and breakdown of the microbial loop to top-down control by unusually large

numbers of protistan bacterivores and to a lack of DOC exudation by phytoplankton. Similar mechanisms may exist in the upper water column of the eastern Ross Sea in late austral summer to early fall.

Conversely, the positive relationship between algae and bacteria in the sea ice suggests that the microbial loop is functioning in the Ross Sea ecosystem. The algae-bacteria association in the sea ice is considerably stronger (1.9-6.6 fold) than that observed by Cota *et al*. (1990) for bacterioplankton in the Weddell Sea. The association is more similar to that shown by Bird and Kalff (1984) for sub-polar fresh and marine waters where bacteria and algae are thought to be strongly coupled. The tight linkage between algae and bacteria in the eastern Ross Sea sea ice and the geographic and seasonal variability in the strength of this linkage in other ecosystems invite reexamination of the factors thought to drive the microbial loop in aquatic habitats.

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Viruses infecting algae and protozoans in Ross Sea winter ice communities

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Both grazing and the sinking of cells and particles are recognized as important in controlling carbon fluxes in the oceans. In lower latitude waters, viral lysis of phytoplankton blooms can also influence carbon flux (see review of marine viruses by Fuhrman 1999); nothing is known of this process in polar regions. Sea-ice communities should be excellent sites for studying viral control of populations of eukaryotic algae and protozoans because populations can reach high densities and many habitats are nearly closed for parts of the year. Viruses have been reported from arctic sea ice (Maranger, Bird, and Juniper 1994), from antarctic waters (reviewed by Karl, Christian, and Dore 1996), and most recently from antarctic lakes (Kepner, Wharton, and Suttle 1998). Although the majority of the viruses enumerated in those studies were thought to be bacteriophages, the authors noted that some could have been viruses of algae or protozoans, especially in the lake samples.

Viruses of both prokaryotes and eukaryotes are potential prey for microheterotrophs. Protozooplankton from natural antarctic populations have been observed with probable individually ingested virus-like particles in their food vacuoles (Gowing 1993). Viruses released from infected cells of pack ice communities could be a food source for grazers.

Objectives of our study were to determine the degree that algae and protozoans of ice communities are infected by viruses and the extent of ingestion of viruses by sea ice protozooplankton. Here, we give an overview of some of the results from our winter cruise. In winter, antarctic pack ice communities with potential for viral control are

- organisms harvested from the water column when ice forms and
- internal communities in young ice with remnants of an autumn bloom.

We sampled sea ice and brine at 51 stations in the Ross Sea from 66°S to 78°S between 171°E and 175°W from 9 May until 11 June 1998. In the field, ice cores were split into 10- or 20-centimeter (cm) sections and then split longitudinally. Onboard ship one half of each section was placed in buffered paraformaldehyde fixative and melted at room temperature ("fast melt"). The other half was placed in filtered seawater and melted slowly over 2 days at 2°C ("slow melt") before fixation. Brine samples were fixed immediately after collection.

We examined thin sections of bulk community preparations with transmission electron microscopy to determine which algae and protozoans were infected with viruses (slow melt aliquots) and to detect grazing on viruses by microheterotrophs (fast melt aliquots). We also enumerated viruses outside organisms in samples ultracentrifuged onto grids for transmission electron microscopy. We differentiated viruses of eukaryotic organisms from bacterial viruses on the conservative ultrastructural criteria of an icosahedral or rounded shape, dense staining,

and capsid diameter $\geq$ 110 nanometers (nm).

Viruses that most likely are infecting algae and protozoans occurred in ultracentrifuge preparations from 23 core sections and 2 brine samples and in thin sections of 9 core sections and 4 brine samples at the 12 stations listed in table. Thus, by inference, infected organisms occur in sea ice communities over a wide latitudinal range in the winter, as well as over time from May through June. A reservoir of eukaryotic viruses exists prior to the summer when many ice algae and protozoans will bloom. Examples of these viruses from the ultracentrifuge preparations are shown in figure 1. Highest abundances of probable eukaryotic viruses were seen in the brine sample from station 159 (figure 1b). The occurrence of a high abundance of what appeared to be the same virus in this sample is consistent with the recent lysis of an infected cell.

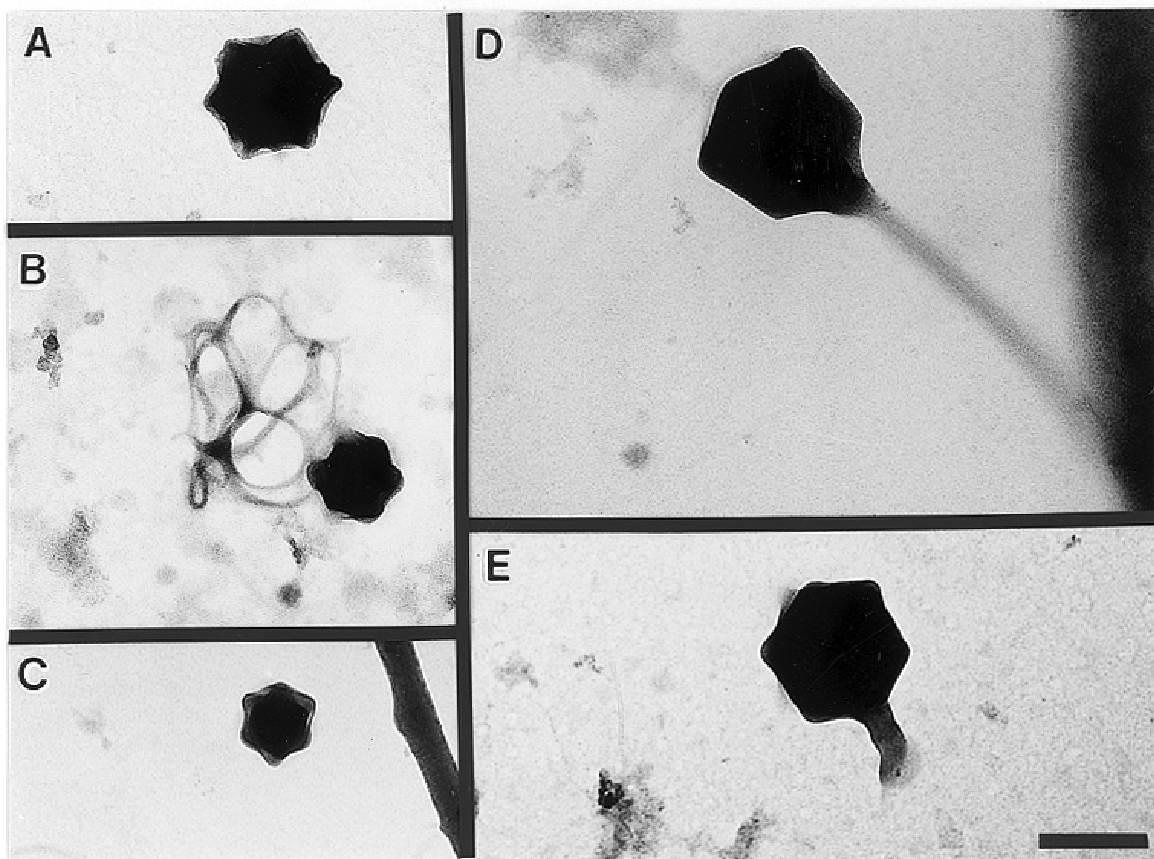

Figure 1. Whole mounts of viruses probably infecting algae and protozoans of ice communities. A. St. 133, 27.5-47.5 cm depth in a 63.5 cm ice core. B. St. 159, brine. An algal scale is next to the virus. C. St. 134, 57.5-69.5 cm depth in a 77.5 cm ice core. D. Tailed virus from St. 159, 64-84 cm section in an 84 cm ice core. E. Tailed virus from St. 134, 17.5-37.5 cm section in a 77.5 cm ice core. Scale bar is 200 nm and applies to A through E.

Stations where probable viruses of ice community eukaryotes occurred.

Station	Location	Sampling Date	Sample type
130-1	68.1°S, 179.9°W	10 May 1998	Core, Brine
130-2	68.2°S, 180.0°W	10 May 1998	Core
131	69.1°S, 179.9°W	11 May 1998	Core
133	71.0°S, 180.0°W	13 May 1998	Core
134	72.0°S, 180.0°W	14 May 1998	Core, Brine
140	76.0°S, 179.9°W	20 May 1998	Core
144	75.9°S, 175.0°E	24 May 1998	Brine
146	75.0°S, 170.9°E	26 May 1998	Core
154	71.9°S, 174.9°E	3 June 1998	Core
157	70.5°S, 177.0°W	6 June 1998	Core
159	69.0°S, 175.2°W	8 June 1998	Core, Brine
162	66.0°S, 175.1°W	11 June 1998	Core

We have not observed any infected cells, although the loose viruses in the samples indicate that infections occurred. It is likely that infected cells are present in low abundances, and thus we need to look at more cells. In lower latitude plankton blooms percentages of infected cells vary from <1 to 50% and are commonly <10%. Many of the samples that we have examined have contained 20 or fewer cells of certain groups of organisms. Some of our samples have contained hundreds of diatom cells, yet we have observed no infected cells

Viruses occurred in thin sections of bulk-embedded material from 9 stations; examples are shown in figure 2. Although we observed nano- and microheterotrophs with food vacuoles, none contained viruses. In all cases, the viruses were either in a fecal pellet with a boundary or in a detrital matrix. Most of the fecal pellets were probably produced by protozoans, although a few contained pulverized silica, suggesting a metazoan producer. Several of the viruses appeared to have been digested (figure 2d), as only their outer boundaries remained. Thus, viruses do appear to be being used as prey in the winter. Some of the viruses were quite large (345 to 545 nm, e.g., figure 1d, 2c) and thus are LVLPs or "large virus-like particles" of the types reported by Bratbak et al. (1992) and Gowing (1993). The host of these cosmopolitan (Gowing 1993) LVLPs remains unknown.

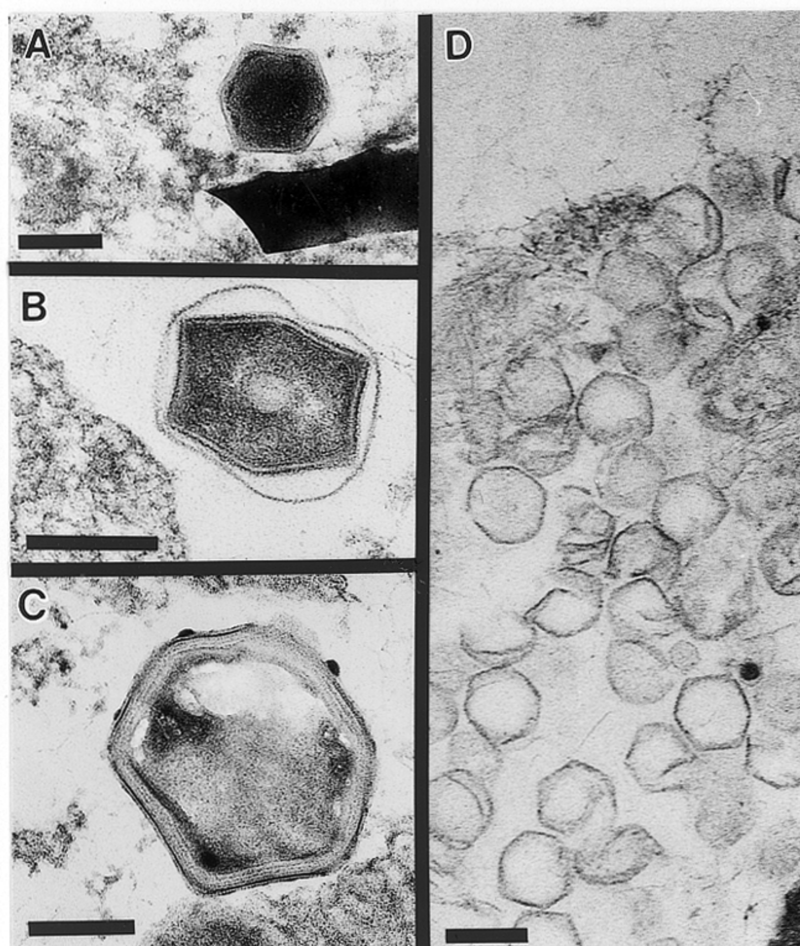

Figure 2. Thin sections of probable algal and protozoan viruses from fecal pellets and detritus. All scale bars are 200 nm. A. St. 129, 38-47 cm section from a 47 cm ice core. B. St. 140, 52-73 cm section from a 73 cm ice core. C. Large (545 nm) virus from St. 159 brine. D. Viruses lacking contents (possibly digested) from St. 146, 67-83 cm section from a 126 cm ice core.

We thank Chief Scientist Martin O. Jeffries, the captain and crew of the research ship *Nathaniel B. Palmer* and Janet Barnes and the Antarctic Support Associates team for making the cruise a success. We thank Martin O. Jeffries and Jean-Louis Tison for help with ice coring, Paula Sicurello and Reena Zalpuri for thin sectioning and Laurie C. Van De Werfhorst for laboratory help. This research was supported by National Science Foundation grant OPP 97-25136.

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Secondary metabolite biosynthesis at ambient temperature in an antarctic sponge-derived bacterium, *Pseudomonas aeruginosa*

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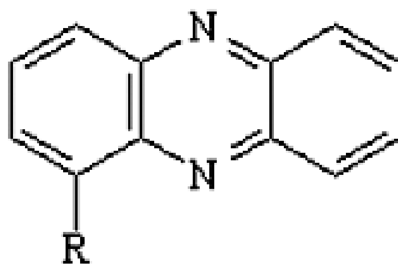
Chemical interactions can be important in mediating biotic interactions at all levels of community organization. Competition for scarce resources and protection from predation, among other ecological phenomena, may encourage the synthesis of bioactive chemical substances. Marine invertebrate-associated microorganisms have received recent attention, due to a variety of potential relationships with their invertebrate hosts. Scientists have speculated that that some secondary metabolites, ascribed to a marine invertebrate, may actually originate in associated microflora (Baker 1996). Sponge-associated bacteria have been shown in some cases to occupy as much as 60 percent of the mesohyl layer—a volume greater than the sponge cells themselves (Santavym Whillans, and Colwell 1990). This suggests that bacteria potentially play important roles in the ecophysiology of the sponge.

Little is known about production of chemical defenses in antarctic invertebrate-associated microorganisms (McClintock and Baker 1997). As part of our ongoing investigation of chemical ecology on the benthos of McMurdo Sound, Antarctica, we have developed a collection of invertebrate-associated microorganisms. To understand the ecology of these organisms and their chemistry, we first focused on characterizing the microbes and their chemistry. This effort resulted in a report on the chemistry from the sponge-associated microbe *Pseudomonas aeruginosa* grown at supra-ambient temperatures (Jayatilake et al. 1996). We report here more recent efforts to define the ecological characteristics of this microbe under more natural thermal conditions.

Isodictya setifera is a bioactive antarctic sponge (McClintock et al. 1994; McClintock and Baker 1997) from which seven different species of bacteria have been cultured (Thornton 1995). One of these microbes, identified as a strain of *P. aeruginosa*, exhibited strong antibacterial activity in bioassays. Fermenting this microbe culture broth under our standard culture conditions isolated six diketopiperazines and two antimicrobial phenazine alkaloids (figure 1; Jayatilake et al. 1996). Of interest to our ongoing studies of microbial chemical ecology are the culture parameters that characterize production of these bioactive alkaloids in *P. aeruginosa*.



(1) R= -CO_2H

(2) R= -CNO_2H

Figure 1. Chemical structures of phenazine alkaloids (1-2).

P. aeruginosa was streaked for isolation onto marine agar and incubated for 48 hours at 30º C. An isolated colony was picked from this plate, inoculated into 100 milliliters Difcomarine broth, and incubated at 30º C with shaking (125 RPM) for 24 hours. One milliliter of this 24-hour culture was used to inoculate each of ten 100-milliliter cultures of marine broth. Five cultures were incubated at 25º C without shaking for 1 week, and the other five at 0º C without shaking for 4 weeks, until all were well into the stationary phase.

To prepare crude extracts for thin layer chromatography (TLC), we pelleted the cells from each stationary phase culture by centrifugation. The supernatants were then filtered through a 0.2 micrometer membrane filter. The 500 milliliters of culture filtrate from the 25º C cultures was passed through an Amberlite XAD-2 column (10 x 2.5 centimeters) twice, and the column was then eluted twice with 300 ml 80% methanol in water. This step was repeated using the 500 ml of culture filtrate from the 0E C cultures. The 80 percent methanol fractions from XAD-2 (0º C and 25º C) were concentrated *in vacuo*, leaving a yellow-beige colored residue. These fractions were then freeze dried and stored at 4E C until used for TLC.

Thin layer chromatography was performed on Whatman reverse phase TLC plates (20 cm x 20 cm). Previously high-pressure liquid chromatography (HPLC)-purified preparations of the phenazine antibiotics (see figure 1) phenazine-1-carboxylic acid (1) and phenazine-1-carboxamide (2), prepared from cultures grown at 25º C, were used as TLC controls. Approximately 1 mg of each control, dissolved in methanol, and 10 mg of each 80 percent methanol/water XAD-2 fraction, also dissolved in methanol, were analyzed by TLC using 5 percent methanol in chloroform. The fully developed TLC plates were visualized using ultraviolet (UV) light at 254 nanometers.

Clearly defined spots can be seen on the chromatogram (figure 2; see arrow) at the site of the phenazine antibiotic controls [phenazine-1-carboxylic acid (1) and phenazine-1-carboxamide (2)] when viewed under UV light. A very faint and diffuse corresponding spot can be seen at

approximately the same location for the fraction collected from the room temperature cultures, confirming that the phenazine antibiotics can be visualized using TLC. A faint and diffuse spot corresponding to the location of the phenazines on the TLC plate for both the controls and the 25º C culture can be seen for the culture grown at 0º C, indicating that phenazine antibiotic production by *P. aeruginosa* is possible at the temperatures encountered in its natural environment in the Antarctic. Because these TLC analyses lack the necessary resolution to separate such closely eluting compounds, it is unknown at this time whether the carboxylic acid 1, the carboxamide 2, or both are being produced by the bacterium at 0º C. Characterizing these extracts using silica gel column chromatography and reversed-phase HPLC from 0º C grown stationary phase cultures would provide additional insights into the specific nature of these compounds.

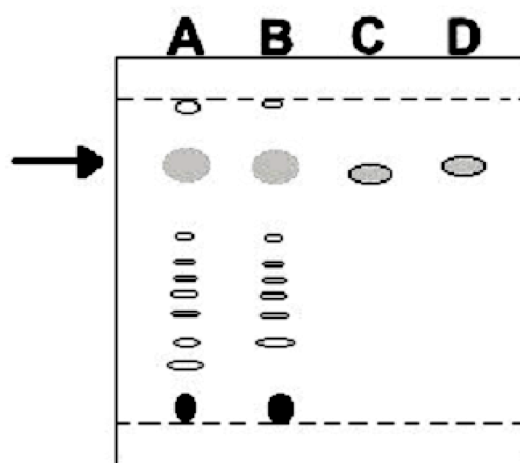


Figure 2. Thin-layer chromatography diagrams of phenazine antibiotic extracts from **Pseudomonas aeruginosa** ***cultures. (A) Cultures grown at 25E C, (B) Cultures grown at 0E C, (C) Purified phenazine-1-carboxamide as a Control, (D) Purified phenazine-1-carboxylic Acid as a Control.***

P. aeruginosa is a cosmopolitan species and this strain grows well at non-antarctic temperatures. It may therefore produce compounds at warmer temperatures that would not be produced at antarctic temperatures. Our results indicate that phenazine alkaloids can be produced at ecologically relevant temperatures. They may, therefore, play a role in the chemical ecology of the antarctic sponge *I. setifera*.

We are grateful to M.P. Thornton, A.C. Leonard and J.E. Grimwade for isolation of the bacterial strain. This work was supported by National Science Foundation grants OPP 95-26610 (BJB) and OPP 95-30735 (JBM), and by logistics provided by the Antarctic Support Associates and the U.S. Naval Support Force Antarctica.

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The temporal cycle of phytoplankton biomass and primary productivity in the Ross Sea, Antarctica

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The Ross Sea biology is abundant. Phytoplankton grow actively and biogenic material accumulates. Chlorophyll concentrations reached at the seasonal maximum are similar to highly eutrophic coastal systems. In such system, carbon undergoes considerable flux among the inorganic, dissolved organic and particulate organic phases. Despite this behavior, few if any Ross Sea investigations have been able to adequately resolve the seasonal patterns of phytoplankton growth. The constraints are largely logistical—ice cover and the availability of ships. Recently, AESOPS (Antarctic Southern Ocean Process Study), a component of the U.S. Joint Global Ocean Flux Study (JGOFS), completed a number of cruises to the Ross Sea. For the first time, the temporal cycle of phytoplankton biomass and productivity can be accurately assessed. Primary data were collected in the southern Ross Sea along 76° 30'S. In addition, two cruises from 1994 and 1995 (NBP94-06 and NBP95-08, part of the Ross Sea Polynya Project or RSP^2) in the same region also are included in the analysis, as the methods used to measure biomass and productivity were nearly identical.

Phytoplankton biomass exhibits a unimodal peak, as has been predicted previously (Cushing 1981; Smith and Sakshaug 1990), with maximum depth-integrated chlorophyll concentrations of 377 mg m^{-2} occurring on year-day 370 (5 January). Biomass is extremely low in early October, increasing rapidly to the seasonal maximum in early January (figure 1). The subsequent decreases during late austral summer and autumn are less rapid, but by year-day 470 (15 April) biomass has declined to its winter (background) levels (less than 10 mg m^{-2}). Hence, active growth and biologically mediated carbon transformations are limited to 6 months in the Ross Sea.

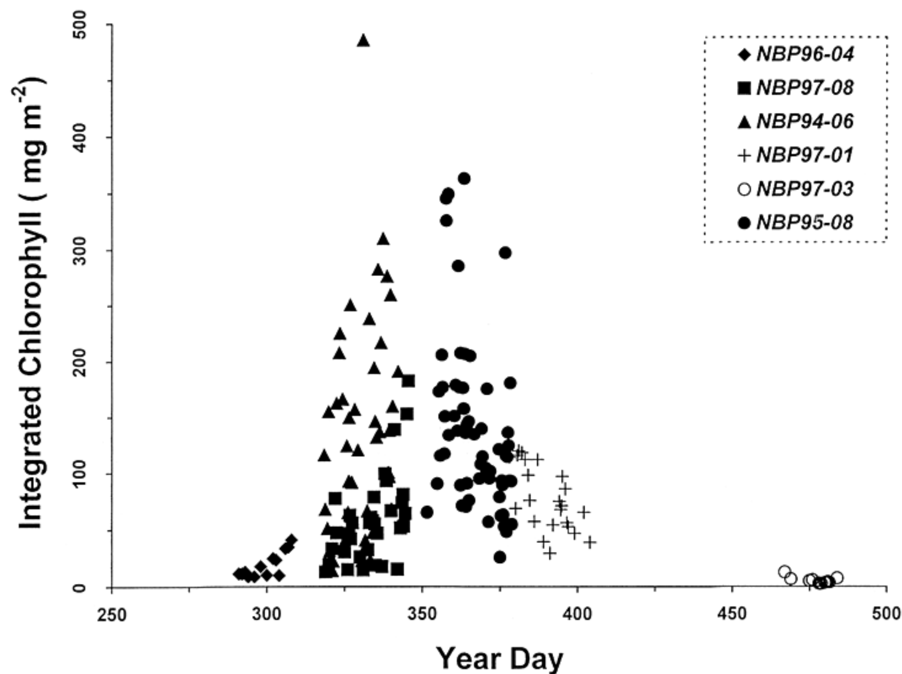

Figure 1. The temporal cycle of integrated (through 100 m) chlorophyll in surface waters of the Ross Sea. Data collected during four AESOPS (NBP96-04, NBP97-01, NBP97-03, and NBP97-08) and two RSP^2 cruises (NBP94-06 and NBP95-08).

Primary productivity—the rate of inorganic carbon fixation via photosynthesis—begins in October and peaks in early December (year-day 343, or 8 December; see figure 2), with mean weekly productivity values reaching 88 mmol C m^{-2} d^{-1}. Thus, the seasonal productivity maximum occurs approximately 3 weeks earlier than the biomass maximum. Furthermore, productivity declines rapidly after the seasonal maximum, despite the favorable irradiance conditions in austral summer.

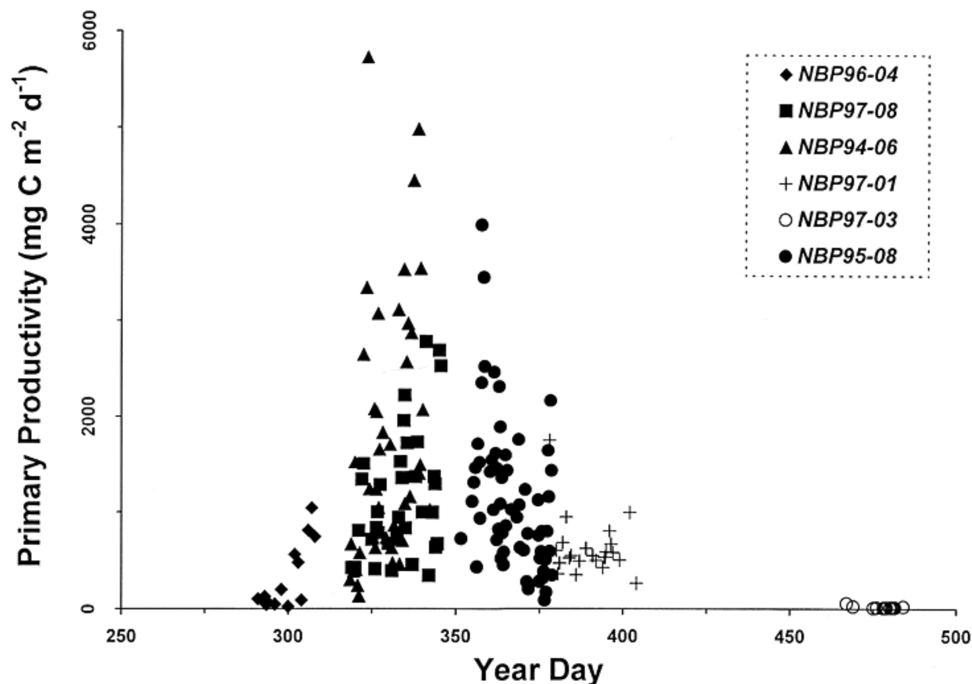

Figure 2. The temporal cycle of integrated euphotic-zone daily primary productivity in the Ross Sea. Data collected during four AESOPS and two RSP² cruises.

These observations support two conclusions:

- First, phytoplankton growth in austral spring is essentially close to the temperature-limited maximum predicted by Eppley (1972). The growth rates we observed (calculated as the change of carbon per unit of living phytoplankton biomass per unit time) during the maximum period of productivity approach 0.5 d^{-1}. The predicted limit was 0.52 d^{-1}. Since net growth rates and short-term growth rates are similar, losses to grazing, remineralization and vertical flux during the austral spring are predicted to be minor.
- Second, productivity (and hence growth rates) declines abruptly during December. Therefore, we suggest that some factor other than irradiance is limiting phytoplankton growth at this time. We hypothesize that trace metal micronutrient concentrations become limiting, since macronutrient concentrations remain replete when growth-rate decreases. Experimental evidence confirms this hypothesis (Coale et al., in preparation; Olson and Sosik, in preparation).

Understanding of the seasonal patterns of biomass and productivity will contribute to more detailed investigations of carbon dynamics. For example, as we now know that growth during austral summer is relatively small, biomass declines in austral summer will largely be controlled by loss processes. Programs designed to assess those loss processes—such as removal by grazing, or the production of larger particles via aggregation and subsequent enhanced

sinking—can now be reasonably conducted, since the timing of the losses is more tightly constrained. Also, additional studies of the details of various aspects of growth that are restricted to shorter periods (for example, the initiation of the bloom in October) are now feasible, as are comparisons across years and with other regions.

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Onset of the *Phaeocystis* Bloom in the Ross Sea: Flagellate versus colony abundance and biomass

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The *Haptophyte Phaeocystis antarctica* is one of the main species that develops in the Ross Sea as part of the massive, seasonal phytoplankton bloom (Smith and Gordon 1997). *Phaeocystis* is a ubiquitous species presenting a complex life cycle (Rousseau et al. 1994), yet its occurrence in natural conditions remains poorly documented — especially whether single cells (flagellated or not) or colonies contribute more to the total *Phaeocystis* carbon (hereafter reported as C) biomass. Even though field observations show that *Phaeocystis* blooms in its colonial form, we have very little, if any, details about how *Phaeocystis'* growth initiates during pre-bloom conditions. Here, we present some preliminary evidences of major changes in the *Phaeocystis* population per se, with particular emphasis on the shift from a single-cell (flagellated or not) to a non-motile colonial stage.

Water samples were taken along the 76°30' S line between 170°E and 178°W during the first Antarctic Environment Southern Ocean Process Study (AESOPS) in the Ross Sea (18 October – 6 November 1996) as part of the U.S. Joint Global Ocean Flux Study (JGOFS) program. The transect was repeated three times from west to east, allowing us to reoccupy some stations several times. The aim of this early-spring cruise was to investigate the initiation of phytoplankton growth in relation to environmental physico-chemical conditions (irradiance, temperature, nutrients). We took fluorometric measurements of chlorophyll-*a* (chl-*a*) in freshly collected water samples and made microscopic observations (epifluorescence and inverted microscopy) of preserved surface water samples (for methodological details (Mathot et al. 2000). Briefly, single cells were enumerated and sized, and cell carbon was estimated by applying a conversion factor of 3.33 pg C per single cell biovolume (expressed in μm^3). Phaeocystis colony carbon was calculated as the sum of colonial cell carbon and a mucus-related carbon term. For this purpose, colonies were counted and measured, and colonial cells were enumerated for a large number of individual colonies, as reported in figure 1. To calculate the colonial-cell carbon contribution, we used the power relationship obtained between colonial biovolume and colony cell number, supplemented by a conversion factor of 13.60 pg C per colonial cell biovolume. On the other hand, the mucus-related carbon was estimated to be 300 ng C per colonial biovolume (expressed in mm^3). Results of these calculations are presented in the table for two locations, revisited at least three times along the time frame of Process 1 (stations 106, 114, 120 at location 'O', 176°E and stations 103, 111, 117 at location 'Orca', 178°W).

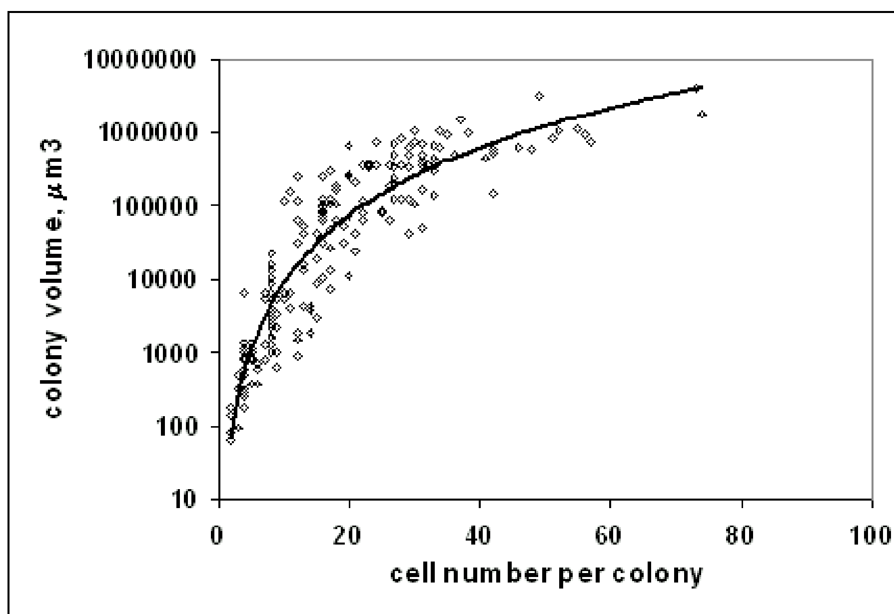

Figure 1. Relationship between Phaeocystis colony volume and cell number per colony. Equation of the fitting curve: Colonial biovolume V = 8.06 [cell number]$^{3.05}$ (R^2 = 0.92).

Phaeocystis ***single cells and colony abundance and biomass at location 'Orca' (76°30'S - 178°W) and location 'O' (76°30'S - 176°E).***

Location & station number	**Local date**	**Single cells**		**Colonies**		
		Abundance, Cells l-1	***Biomass, µgC l-1***	***Abundance, Col.Cells l-1***	***Col. Cell Biomass, µgC l-1***	***Mucus Biomass, µgC l-1***
Orca						
103	19-Oct	4.20E+05	1.40	2.31E+04	0.31	0.006
113	29-Oct	1.15E+05	0.38	1.98E+05	2.69	0.059
117	3-Nov	9.00E+04	0.30	3.86E+05	5.25	0.390
O						
106	21-Oct	5.29E+05	1.76	2.68E+04	0.36	0.010
114	30-Oct	3.01E+05	1.00	3.33E+05	4.53	0.028
120	5-Nov	4.57E+05	1.52	9.49E+05	12.91	0.773

Surface phytoplankton biomass was overall very low during the first transect, rarely exceeding 0.1 µg chl-a l^{-1} (figure 2). By the third transect, overall phytoplankton biomass had

increased to about 0.7 µg chl-a l^{-1} and peaked up to 1.0 µg chl-a l^{-1} at one location ('Z', station 121). At the beginning of the cruise, we observed *Phaeocystis* mostly as single cells (95 percent of the total *Phaeocystis* cell abundance; table, stations 103 and 106) with an average equivalent spherical diameter (ESD) of 3.1 µm (range = 2.4-5.5 µm). This pool of single cells comprised both flagellated cells and other cells that were clearly devoid of flagella but not yet part of a colony. During this pre-bloom period, *Phaeocystis* had begun to form small round colonies (ESD ~10-150 µm), which we detected—although in very low number—during the first occupation of the 76°30' transect. The colonies clearly appeared as two-celled first, then groups of 4, 8, and more cells embedded in a thin but distinct mucous envelope.

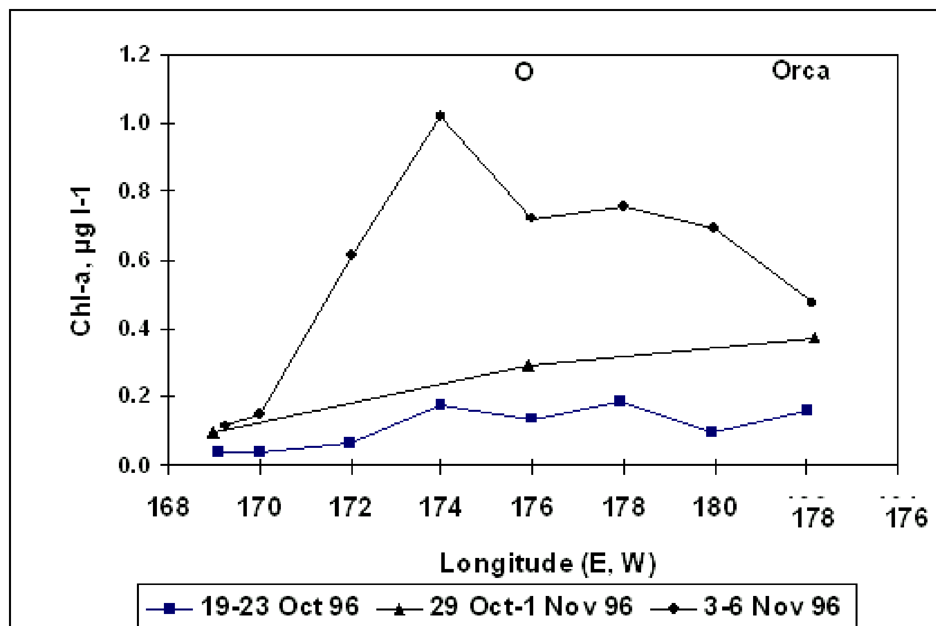

Figure 2. Surface phytoplankton biomass distribution (expressed as µg Chl-a l^{-1}) along 76°30' of latitude South between longitudes 170° East and 178° West.

The general trend of *Phaeocystis* biomass changes within 15 days between the first and the last reoccupation at both "O" and "Orca" was similar (table). However, this biomass increase was more pronounced in "O" than in "Orca," due to the "take off" of the colonial form at station 120 (13.68 µg C l^{-1}) as compared to station 117 (5.64 µg C l^{-1}). Assuming that there is no or negligible removal processes (such as grazing and/or sedimentation) during this early-spring period, we estimated the specific *Phaeocystis* growth rate to be twice as high at location "O" than at "Orca." These differences between "O" and "Orca" in the timing of the appearance of the colonial form should be viewed both temporally and spatially. During the Process 1, ice conditions in the Ross Sea polynya region were heavy (10/10), as observed from satellite images of ice coverage. By the time of the last transect, however, the polynya had started to open from south to north. Although the ice cover was still almost complete (8-9/10), ice was thinner and allowed some irradiance to penetrate in the water column, earlier at "O" than at "Orca."

From these data, we conclude that the initiation of the *Phaeocystis* bloom might be

triggered—but, we believe, not solely—by the amount of irradiance penetrating the water column under ice conditions, still heavy in October and November in the Ross Sea area. The stabilization of a shallow, upper mixed layer did not seem to affect the bloom initiation per se (though it could affect the bloom's magnitude and extension), as the water column remained well mixed (70 meters or more) all through Process 1 cruise.

Understanding the partitioning of carbon within *Phaeocystis* will provide insights into the potential role of the microbial food web (and related transformations within the carbon pools) in the surface layer of antarctic waters, as well as the potential fate of *Phaeocystis* biomass as they settle and are exported in the form of settling exported particles. Clearly, *Phaeocystis* colonies play a significant role with respect to the overall carbon and energy circulation pattern. The question about why *Phaeocystis* is so successful still remains open. The forming of colonies during early stages of its life cycle could be a strategy for *Phaeocystis* to avoid death by predation.

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AESOPS: Effects of iron addition on nutrient depletion and nitrogen uptake rates in an offshore region of the Ross Sea

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Large sections of the Southern Ocean are characterized as high nutrient, low chlorophyll regions (HNLC). These waters have relatively high concentrations of the macronutrients, nitrogen and phosphorus, yet rates of biomass accumulations and primary productivity remain low. Results of iron-enrichment bottle experiments in the HNLC waters of the Southern Ocean suggest that phytoplankton growth may be limited by iron, a major component of many enzymes, pigments, and DNA-related proteins in phytoplankton (e.g. Martin et al. 1990; 1991; de Baar et al. 1995). Relative to the macronutrients, the concentration of iron in many parts of the region is well below that required by phytoplankton (Martin et al. 1990).

Our objective was to determine the effects of iron additions on rates of nitrogen uptake. Nitrate uptake requires iron and this requirement may explain the inability of phytoplankton to use the relatively high concentrations of $NO3^-$ present in HNLC areas where concentrations of iron are very low.

We performed experiments at an offshore site (Station Blue; 74° 20' S, 176° W) in the Ross Sea during January-February 1997 aboard the U.S. Antarctic Research Program research ship *Nathaniel B. Palmer* as part of the U.S. Joint Global Ocean Flux Study, Southern Ocean program. Samples were collected from 25m and incubated with varying additions of iron for 14 days in 20 L carboys; sample collection and subsequent experimental manipulations followed trace metal clean protocols (Fitzwater et al. 1982). Iron was added to the 20 L carboys to give final concentrations of 0.2, 0.5, 1.0, and 2.5 nM; two carboys with no iron additions were used as controls. Carboys were incubated at *in situ* temperature and light in on-deck Plexiglas® incubators.

Concentrations of nitrate (NO_3^-), ammonium (NH_4^+), particulate nitrogen (PN), chlorophyll *a* (chl *a*), phosphate (PO_4^{-3}), and silicate (SiO_2) were monitored in each carboy throughout the study. Nutrient concentrations ($NO_3^{-,}$ PO_4^{-3}, and SiO_2) were determined using a Technicon AutoAnalyzer II (Parsons et al. 1984), NH_4^+ was measured with the manual phenol hypochlorite method (Solorzano, 1969), and chl *a* concentrations were determined spectrophotometrically after a 90% acetone extraction for about 24 hours in a freezer.

Subsamples were withdrawn from each carboy four times during the study and uptake rate of NH_4^+ and $NO_3^{-,}$ by both the whole water and the >5 µm fraction, were determined using ^{15}N tracer techniques. Samples for ^{15}N analysis were collected by filtration (<80 mm Hg) onto

precombusted Whatman GF/F filters and 5.0 µm silver filters, and analyzed mass spectrometrically (Dugdale and Goering 1967).

Ambient concentrations of the major nutrients were high in the study area (figure 1). The concentrations of NO_3^-, NH_4^+, and PO_4^{-3} all decreased during the study (figure 1); the larger the iron enrichments, the greater the decrease in NO_3^- concentrations. Increasing additions of iron resulted in increasing amounts of nitrogen being incorporated into the PN, and there was a strong inverse correlation between NO_3^- and chl *a* concentrations ($r^{-2} = 0.974$, data not shown).

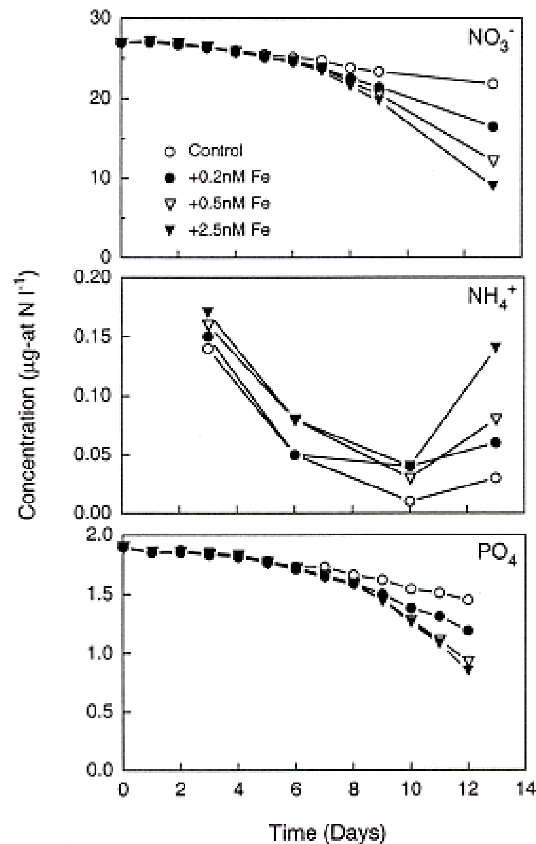

Figure 1. Macronutrient concentrations in the control carboy and treatment carboys versus time in days.

The greater the iron enrichment, the higher the uptake rates of both NO_3^- and NH_4^+ (figure 2). The bulk of the increases in uptake of both NO_3^- and NH_4^+ was attributed to organisms > 5.0 µm in size (figure 2).

Nitrogen concentrations within the carboys declined when iron was added. This observation suggests that nitrogen uptake at this offshore site in the Ross Sea was limited by iron. Only small decreases (figure 2) in NO_3^- concentrations occurred early in the experiment compared to the high initial depletion of NH_4^+, possibly due to preferential use of more reduced forms of nitrogen. As concentrations of NH_4^+ approached the limit of detection, concentrations of NO_3^- began to decrease at a higher rate.

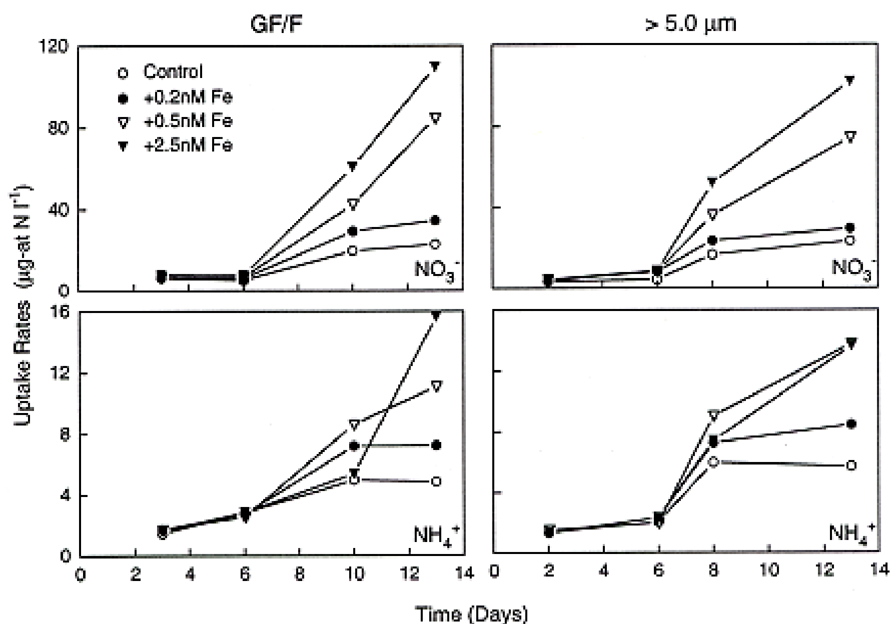

Figure 2. Rates of NO_3^- and NH_4^+ uptake of both the GF/F and > 5.0 µm fraction versus time in the control and treatment carboys

The addition of iron resulted in large increases in nitrogen uptake rates. Additions of 2.5 nM iron showed a five-fold increase in NO_3^- uptake and a three-fold increase in NH_4^+ uptake over uptake rates in the controls. In an earlier study, experiments performed in the Ross Sea showed NO_3^- uptake rates in samples fertilized with iron to be three-fold higher than the rates of NO_3^- uptake in control samples containing no iron addition (Martin et al. 1990). While Martin et al. (1990) found that only slight increases in uptake were seen with increasing additions of iron, our results indicate that uptake rates increased significantly with increasing additions of iron. The enrichments likely provided the iron necessary for nitrate and nitrite reductase production, which in turn resulted in the increased NO_3^- uptake rates we observed.

Size fractionation showed that the increased uptake of nitrogen occurred primarily in the >5.0 µm fraction. Earlier studies, in the equatorial Pacific, have shown that areas of low iron concentration are largely dominated by smaller picoplankton species presumably because the low iron concentrations are limiting to larger, bloom-forming species (Barber and Chavez 1991). Our size fraction data are consistent with this view.

In conclusion, the results of this study are similar to those found in earlier iron limitation studies in the Ross Sea sector of the Southern Ocean as well as other HNLC regions (Martin et al. 1990; Martin et al. 1994). We found that iron additions have a direct effect on nitrogen uptake and nutrient depletion at one site in the Ross Sea. Rates of NO_3^- and NH_4^+ uptake increased over non-enriched samples as concentrations of iron increased. These ^{iron} additions also resulted in increased chl *a* and PN concentrations. Our data suggests that iron is a limiting factor in nitrogen uptake by phytoplankton in this area of the Southern Ocean. Increases in the

available iron would allow the phytoplankton community in the area to take advantage of the relatively high macronutrient concentrations present.

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AESOPS: Ammonium uptake kinetics in the Ross Sea, Antarctica

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To assess the role of macronutrients in controlling primary productivity in the Southern Ocean, it is necessary to quantify community nitrogenous nutrition over relevant spatial (ice-edge to open ocean) and temporal (seasonal) scales. In the high productivity systems of the Antarctica Marginal Ice Zone (MIZ), the main source of nitrogen supporting phytoplankton production appears to be nitrate (NO_3^-: "new" production; Nelson and Smith 1986; Smith and Nelson 1990; Goeyens et al. 1991). In contrast, the nitrogen demands of phytoplankton in the lower productivity systems found offshore of the continental shelf are met primarily by ammonium (NH_4^+: "regenerated production"), despite the elevated ambient concentrations of NO_3^- present (Glibert et al. 1982; Rönner et al. 1983; Koike et al. 1986).

Seasonal trends of an increasing dependence on regenerated nitrogen forms have been reported for the Scotia Sea (Olson 1980; Glibert et al. 1982; Koike et al. 1986; Owens et al. 1991); Weddell Sea (Kristiansen et al. 1992; Rönner et al. 1983), Scotia-Weddell Confluence area (Goeyens et al. 1991), presumably due to the higher ambient concentration of NH_4^+ during summer and autumn and changes in the species composition of the phytoplankton community. As part of the JGOFS Antarctic Environment Southern Ocean Process Study (AESOPS), we estimated the first NH_4^+ uptake kinetic parameters of the natural planktonic assemblages in the Ross Sea and determined the seasonal variability in their ability to utilize NH_4^+.

A continental shelf station, (*O*; the mid-point of the primary AESOPS transect-line) and a station adjacent to the Ross Ice Shelf (Emperor) were sampled in the Ross Sea, aboard the research ship *Nathaniel B. Palmer* during austral spring and summer (table 1). Discrete samples for shipboard uptake experiments were collected from surface waters (spring: 2 m; summer: 4-5 m) using a trace-metal clean, instrumented rosette with 30-liter Go-Flo bottles. All subsequent sample manipulations were conducted within a laminar-flow hood (HEPA) using trace-metal clean techniques (e.g., Fitzwater et al. 1982).

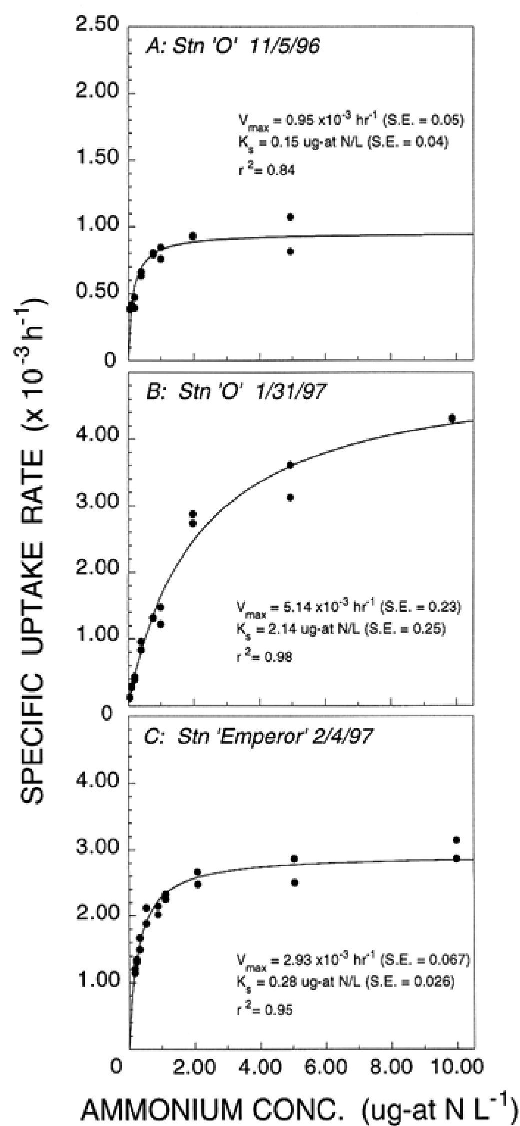
Table 1. Ross Sea study sites for uptake kinetics experiments during austral spring (complete ice-coverage) and summer (open water).

Station name/number	Location	Sampling date	Chlorophyll-a (mg/m^3)	Ambient concentration* NO_3^-	NH_4^+	Urea
O/120	76° 27.74'S 176° 0.90'E	November 5, 1996	0.72	30.47	<0.03	nd
O/217	76° 26.73'S 176° 12.79'E	January 31, 1997	4.10	11.19	<0.03	0.20
Emperor/223	77° 59.52'S 176° 2.99'W	February 4, 1997	1.12	11.78	0.13	0.13

nd = not determined

Concentrations expressed in µg-at N L^{-1}

Nitrogen uptake experiments were conducted in acid-clean, 1.2-L polycarbonate bottles, in duplicate after inoculation with $^{15}NH_4Cl$, (99 atom%; Cambridge Isotopes) at a range of initial substrate concentrations (0.51, 0.10, 0.20, 0.40, 0.75, 0.99, 1.97, 4.93 and 9.86 µg-at N/L. Inoculated bottles were hermetically heat-sealed within polyethylene bags and incubated for either 24 hours (spring) or 12 hours (summer) in Plexiglas® deck incubators at *in situ* temperature and light. Ambient concentrations of nitrate (NO_3^-) were determined with a Technicon® Autoanalyzer II, and NH_4^+ and urea were analyzed manually according to Solórzano (1969) and Price and Harrison (1987), respectively. Samples for ^{15}N analysis were collected by filtration (< 80 mm Hg) on precombusted Whatman GF/F filters (2.5 cm), and frozen for mass spectrometry. Pigment concentrations (chlorophyll *a*) were determined spectrophotometrically after 90% acetone extraction for approximately 24 hours in a freezer. Nitrogen specific uptake rates, estimated from ^{15}N accumulation in the particulate fraction, were calculated using equation number 5 of Dugdale and Wilkerson (1986). Michaelis-Menten parameters: half-saturation constants (K_s), and maximum uptake velocities (V_{max}) were estimated from a direct nonlinear curve fitting model to the Michaelis-Menten equation for enzyme kinetics, $V = V_{max}S/(K_s+S)$, where S is the limiting nutrient (figure).



Nitrogen specific uptake rates of NH_4^+ verses initial concentration of NH_4^+ for natural assemblages from station "O" on November 5 (A) and January 31 (B), and station "Emperor" on February 2 (C). The curved plots are fitted directly to the Michaelis-Menten equation.

Results And Discussion. Kinetic parameters for NH_4^+ uptake indicate that the Ross Sea phytoplankton at station 'O' change dramatically in their ability to use NH_4^+ as the growing season progresses (table 2). From November (complete ice coverage) to January-February (open water), their effective use of low concentrations of NH_4^+ (ambient concentrations rarely exceed 0.2 µg-at N/L in the central Ross Sea during spring-summer) decreases substantially (about 3-fold decline in the initial slope [α] of the Michaelis-Mention plot). However, maximum uptake velocities (V_{max}) of NH_4^+ increase 14 fold during the 12-week interval between sampling periods, indicative of greater potential NH_4^+ uptake capability, provided that elevated (nutrient-sufficient)

concentrations are available for growth.

Table 2. Kinetic parameters for ammonium uptake of natural assemblages of Ross Sea phytoplankton.

Michaelis-Menten parameters: half-saturation constants (K_S), maximum uptake velocities (V_{max}) are estimated from a direct nonlinear curve fitting model to the Michaelis-Menten equation for enzyme kinetics, $V = V_{max}S / (K_S + S)$, where S is the limiting nutrient. The degree of nutrient affinity (α) at low concentrations (i.e., $S< K_S$) is in direct proportion to the magnitude of the initial slope of the Michaelis-Menten plot (i.e., $\alpha = V_{max}/ K_S$; Healey, 1980; Cochlan and Harrison, 1991). Estimated standard error values are given in parentheses.

Station/date	Vmax [x 10-3 h-1]	Ks [µg-at N . L-1]	α [x 103 h-1 (g-at N . L-1) –1]
O 11/05/96	0.953 (0.048)	0.15 (0.035)	6.35
O 1/31/97	5.14 (0.23)	2.14 (0.25)	2.40
Emperor 2/4/97	2.93 (0.067)	0.28 (0.026)	10.5

During both seasons, the species composition of the community remains dominated by the haptophyte *Phaeocystis antarctica*, which becomes colonial during spring (S. Mathot personal communication). The phytoplankton assemblage nearer to the ice-edge sampled in February demonstrated an intermediate V_{max} for NH_4^+ (average of the seasonal progression of estimates from station "O"), but a low half-saturation constant for NH_4^+resulting in the highest affinity (α) for low concentrations of NH_4^+. Thus the microbial community closest to the Ross Ice Shelf, composed of *P. antarctica*, diatoms, dinoflagellates and flagellates, can most effectively sequester the sub-saturating concentrations of NH_4^+ found in this study.

In summary, NH_4 uptake and its "preference" over NO_3^- could be significant in the Ross Sea MIZ, provided the phytoplankton are exposed to elevated concentrations of NH_4^+. However from October to February, ambient concentrations of NH_4^+ are generally very low (< 0.03 – 0.10 µg-at N/L), and the greater ambient concentrations of NO_3^- (9-31 µg-at N/L) provides all the nitrogen required for growth in the central Ross Sea. Nitrate-fueled production ("new production," *sensu* Dugdale and Goering 1967) dominates the central Ross Sea productivity: Ä-ratios [Ä = NO_3^- uptake/(NO_3^- + NH_4^+uptake)] averaged 0.96 at station "O" during both seasons, and Ä = 0.51 at the ice-edge station in the summer.

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Investigations of Weddell seal (*Leptonychotes weddellii*) populations in McMurdo Sound, 1998-2000

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During the 1998-1999 and 1999-2000 field seasons (hereafter referred to as 1998 and 1999 respectively), we continued the studies of Weddell seal (*Leptonychotes weddellii*) population dynamics in McMurdo Sound that began in the late 1960s. In addition to this long-term tagging and mark-recapture program, we focused on determining immigration and emigration rates, quantifying male reproductive success, establishing reference ranges for health parameters, and tracking the seasonal movements of adults and weaned pups.

Weddell seals often return to historical locations each spring to give birth and breed (Testa and Siniff 1987). This annual site fidelity concentrates and reduces their spatial range enabling researchers to perform multiple surveys of the entire population to estimate the population size. From mid-October through late December of each season, we continued the tagging and survey program begun in 1969, adding to a sightings database of individually tagged seals within Erebus Bay, the main study area (figure 1), and the rest of McMurdo Sound. As Weddells are such a long-lived species (some individuals are known to be over 28 years old), we have only recently been able to collect sighting records spanning the entire life of an individual animal.

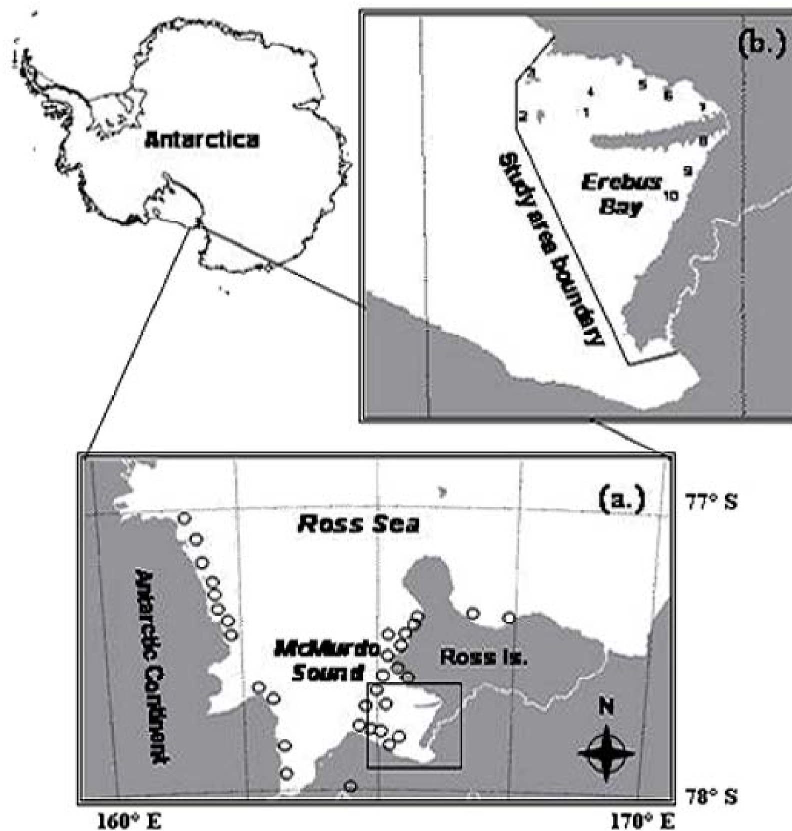

Figure 1. (a.) Map of McMurdo Sound showing where surveys were conducted outside of the main study area for the immigration/emigration study. (b.) Map of Erebus Bay with the boundary of the main study area indicated. Major colonies are marked by the following numbers: 1) Big Razorback, 2) Tent, 3) Inaccessible, 4) Little Razorback, 5) Tryggve Point, 6) Turk's Head, 7) North Base, 8) South Base, 9) Hutton Cliffs, 10) Turtle Rock.

Abundance estimates are calculated using a Jolly-Seber mark-recapture model (Seber 1982) slightly modified by Siniff et al. (1977) for use in studies which are unable to tag every animal seen during a survey. Population estimates are calculated from the ratio of marked to unmarked animals and the frequency of sighting each marked individual. Therefore, the proportion of tagged animals in the population is directly related to the precision of our estimates. Since 1974, efforts have been made to tag very newborn pup seen within the main study area (figure 2), and in recent years we have placed an increased emphasis on re-tagging animals with broken or partially missing tags before the individual seals become "lost" to these analyses. Currently around 80% of the Erebus Bay population is marked. In 1998, we performed 7 surveys and tagged 490 newborn pups inside the study area. The largest pupping colony was Turk's Head (101 pup births). The population estimate for adult males was 250, (SE= 19), and for adult females it was 665, (SE= 14). In 1999, we performed 8 surveys and tagged 498 newborn pups. The largest pupping colony was Hutton Cliffs (99 pup births). The population estimate for adult males was 321, (SE= 18), and for adult females it was 529, (SE= 15).

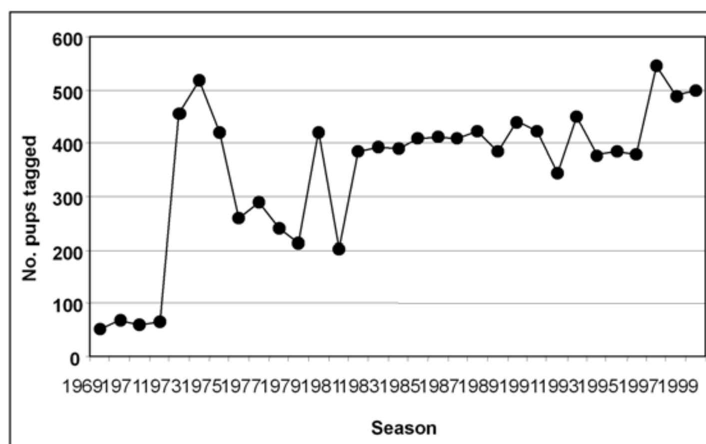

Figure 2. Weddell seal pups tagged in Erebus Bay between 1969-1999. Since 1974 nearly every pup in the study area has been tagged, except in 1981 when approximately only half of the pups were tagged.

The total number of seals tagged and re-tagged from 1969 to 1999 in McMurdo Sound during this long-term research program.

Season	New tags	Re-tagged	Season	New tags	Re-tagged
1969	50	0	1985	567	190
1970	214	0	1986	492	114
1971	329	31	1987	425	133
1972	229	10	1988	439	270
1973	626	4	1989	400	189
1974	706	31	1990	469	182
1975	539	31	1991	428	226
1976	400	22	1992	366	285
1977	581	42	1993	464	155
1978	334	15	1994	380	134
1979	399	66	1995	388	83
1980	581	44	1996	428	94
1981	310	67	1997	800	56
1982	501	203	1998	659	142
1983	525	302	1999	768	171
1984	528	210			

When comparing inter-annual population trends, it is important to consider the rates of immigration and emigration. Previous researchers (Siniff et al. 1977 and Testa 1987) have suggested that immigration into the study area was necessary for the Erebus Bay population to remain stable. During the 1998 and 1999 seasons, we continued to conduct intensive searches for marked seals outside the study area, while at the same time tagging potential immigrants (434 adults in the last three years). Our objective is to estimate the number of adult seals that move into and out of the study area annually, and to determine the importance of seals born outside the study site to the dynamics of the Erebus Bay population. Preliminary results suggest that immigration rates need not be as large as previously thought to maintain a stable population size. Testa (1987) estimated that less than 20% of the adult population was actually born in Erebus Bay, and Hastings (1999) suggested that an estimated survival rate of 12% from birth to age 6 supported Testa's claim. Annual survey data however indicates that at least 60% of the adults (identified by visual estimation as animals older than or equal to two years old) in Erebus Bay were born and tagged there. This proportion continues to increase every year due to the Weddell seals' longevity and retagging efforts, which prevents animals born and tagged in the study area from being mis-identified as immigrants. While some immigration does occur most immigrants originate from colonies around Ross Island, and immigration from colonies across McMurdo Sound seems rare (Cameron 2001).

White Island harbors a population of Weddell seals that has been geographically isolated from the rest of McMurdo Sound by the Ross Ice Shelf since at least the mid-1950's (Heine 1960; Stirling 1972). Surveys of the White Island population, including tagging and re-tagging efforts, have been conducted since 1991. In 1998, we observed and tagged 9 new pups (5 females, 4 males), and in 1999, we tagged six new pups (all females). As a point of reference, the past average annual pup production and proportion of females born is 6.13/year (SD=1.80), and 0.73/year (SD=0.17) respectively. Tissue samples collected from White Island animals will help to clarify the genetic relatedness of seals both within the population and as compared to the McMurdo Sound population. Preliminary results suggest that the White Island seals are indeed genetically different from those in the rest of the Sound.

Studies concerning the behavioral ecology of adult males continued at Big Razorback Island. It has been assumed that Weddell seals are polygynous; however, because they breed underwater within male-defended territories, observing a mating event has been difficult. To better define their mating system we, in collaboration with the laboratory of Curtis Strobeck at the University of Alberta, are using molecular genetics to examine the paternity of pups born to breeding females from Big Razorback Island. In 1999, 40 pups, born to mothers present at Big Razorback in 1998, were sampled for this paternity study. Of these, 30 pups were fathered by one of nine males present during the 1998 season. Initial results suggest that Weddell seals are in fact mildly polygamous, each successful male mating with 1-7 females (Gelatt 2001). Behavioral data, collected with conventional VHF radio transmitters and underwater depth transmitters, indicate that males seem to employ one of two strategies while defending their underwater territory. Most appear to spend the majority of their time patrolling the hole within a few meters of the surface, and occasionally diving to over 300 meters. Others remain inactive below the surface returning occasionally to breathe.

We are also interested in the overall health of the population and for the fourth year we collaborated with scientists from Hubbs-Sea World Research Institute (Drs. Pam Yochem and

Brent Stewart) to establish reference ranges for various health parameters. In previous years we collected blood samples throughout the breeding season (November-December). In 1999 we also took samples in February, after the breeding cycle, to look for temporal effects in their blood chemistry and various health parameters. Most blood parameters were within ranges reported for other phocids; several varied with respect to age (e.g., white blood cell counts, monocyte counts, serum urea nitrogen and creatine were higher in adults; serum glucose, cholesterol, triglycerides, and iron were higher in pups) and condition (e.g., higher red blood cell counts, lower white blood cell counts, and fewer bands and neutrophils in clinically healthy adults) (Yochem et al. 1998). Additionally, as part of an ongoing investigation on the seasonal movements of Weddell seals, in 1998 we instrumented 10 pups and in 1999 we instrumented 10 adults with satellite-linked location transmitters. Records obtained from pups instrumented in 1996 and 1997 indicated that pack-ice habitats may be more important to the ecology and life history of juveniles than previously thought as these individuals spent most of their time within the pack-ice, however without hauling out (Stewart et. al. 1998).

Finally, we continued to collect skulls from adults found dead on the ice for contribution to a reference collection maintained by the National Marine Mammal Laboratory in Seattle, WA. The collection now has one complete skeleton and 51 skulls from all age classes and both sexes. Most of the specimens are from known-aged animals due to the tagging efforts of this project.

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C.L.I.M.A. Project activities and objectives in the Pacific sector of the Southern Ocean

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Italy actively participates in the efforts of the international scientific community in Antarctica through the activities of the P.N.R.A. (the Italian National Programme for Antarctic Research). Part of the Italian antarctic program, the CLIMA Project (Climatic Long-term Interaction for the Mass-balance in Antarctica), has the objective to investigate the role of Antarctica in the global climate change.

CLIMA started as a physical oceanographic project, focusing on the main aspects of large-scale (Antarctic Circumpolar Current) and mesoscale (Ross Sea) circulation in the Pacific sector of the Southern Ocean. Research activities have been focused mostly in the Ross Sea region in past years, to understand the mechanisms of dense water formation and diffusion. However, over time the CLIMA Project has broadened its original objective to include the investigation of aspects of biogeochemical processes and dynamics by using a multidisciplinary approach and chemical and biological tools.

The understanding of the role the Southern Ocean has in global change cannot be separated from the carbon cycle of the Southern Ocean and the processes affecting it. For this reason, the study of biogeochemical fluxes has been undertaken, specifically the fluxes of biogenic elements and some biological components (e.g., phytoplankton, fecal pellets). In addition, the efficiency of the biological pump does not simply consist in the capacity of transforming atmospheric CO_2 into organic matter but in "sequestering" carbon by transferring it to the water where exchange with the atmosphere is hindered for centuries. Consequently, the assessment of gas exchange rates between ocean and atmosphere is of crucial importance. It has become a major focus of the Project, together with the study of carbon transfer through different compartments of the biota (e.g., viruses, bacteria, nanoplankton, microphyto- and microzooplankton).

Two major oceanographic cruises were completed in austral summers 1994-1995 and 1997-1998. En route from New Zealand to the Ross Sea, along the P14S WOCE (World Ocean Circulation Experiment) section, expendable bathythermographs (XBTs) were launched every 5-20 miles to study the hydrological structure of the upper 700 m (figure1). Drifting buoys were concomitantly released at the surface and pop-up floats deployed at about 1,000 m with the aim of describing the pathways followed by the surface and sub-surface currents, the dynamics of the ACC (Antarctic Circumpolar Current) and the variability of front locations. During these oceanographic surveys more than 350 hydrological stations were performed (figure 2), yielding vertical profiles of temperature, conductivity, salinity, pH, dissolved oxygen, transmittance, fluorescence, and PAR (Photosynthetically Active Radiation). Water samples were also collected within the water column for the determination of dissolved oxygen, nutrients and micronutrients, chlorofluorocarbons, dissolved organic carbon, particle size, total suspended matter, particulate

organic carbon and biogenic silica, and pigments. Incubations were carried out to quantify primary production and respiration rates, bacterial metabolic activity, grazing efficiency of nano-, micro- and mesozooplankton, and egg production and hatching success of some dominant copepod species. Sea-surface temperature, meteorology, and ice-cover data were also obtained both *in situ* and remotely sensed observations.

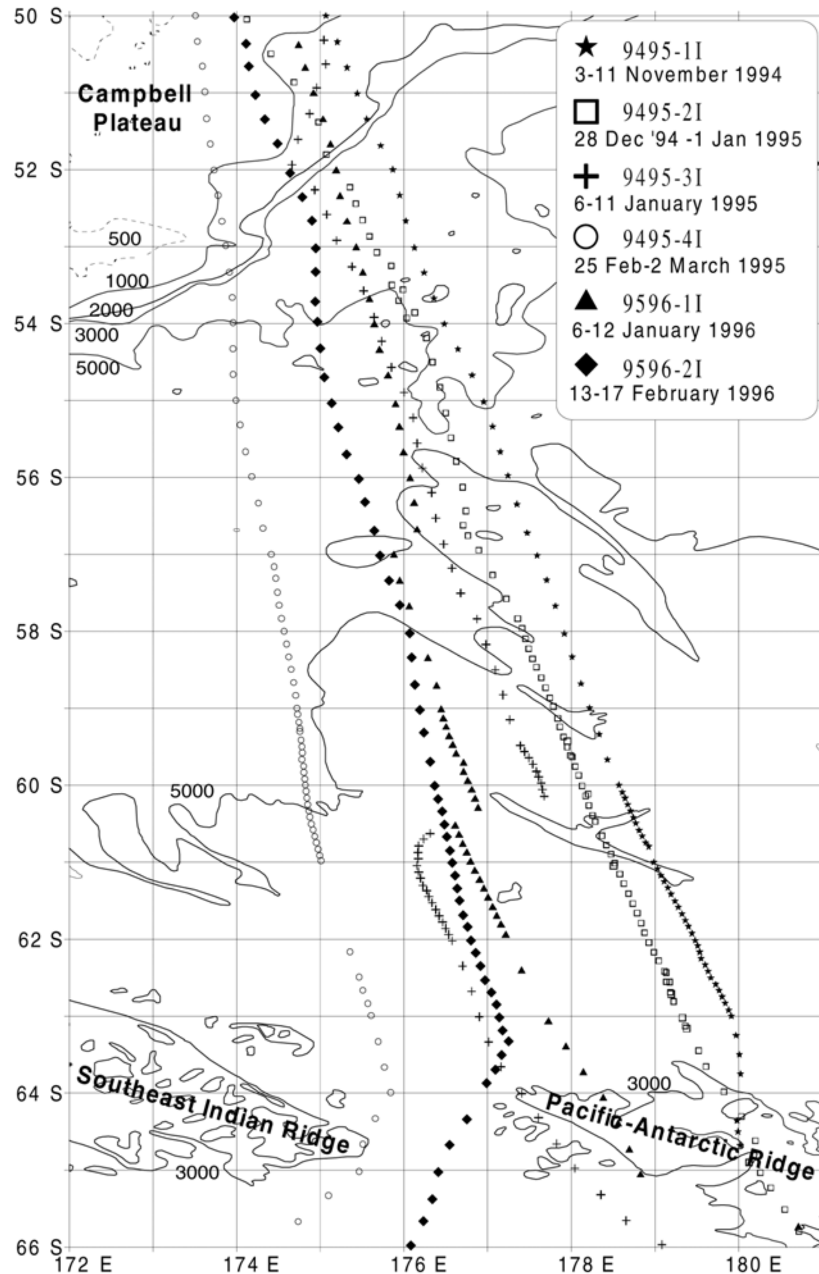

Figure 1. XBTs launched along the route from New Zealand to the Ross Sea.

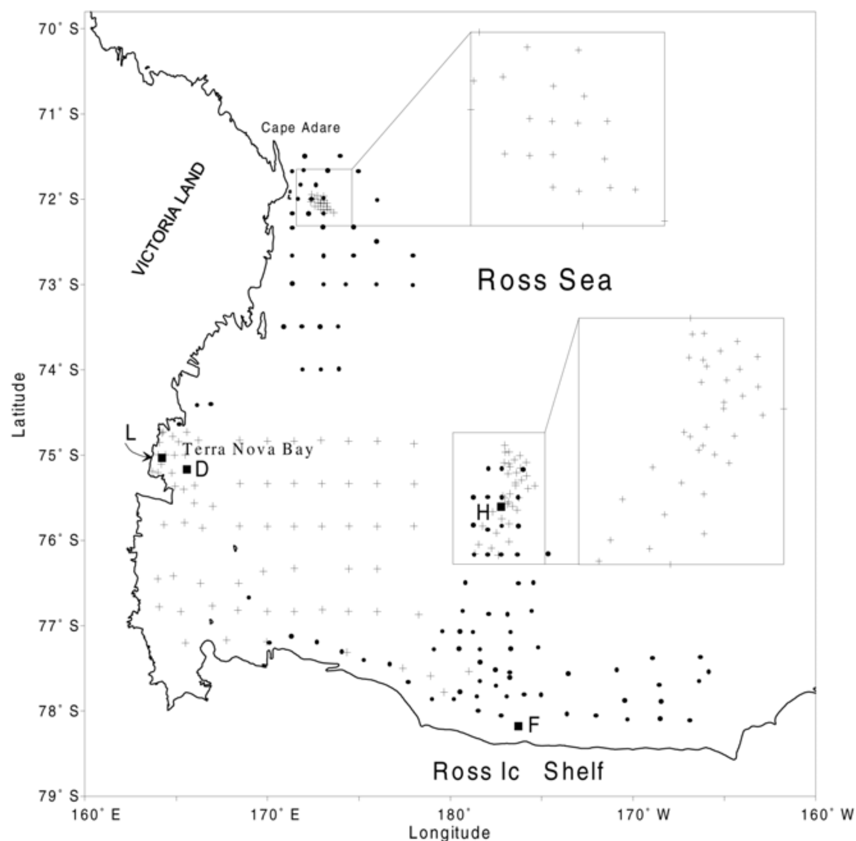

Figure 2. Station location and mooring sites in the Ross Sea. Moorings are represented by capital letters. Circles and crosses indicate hydrological stations performed during the 1994-1995 and 1997-1998 surveys respectively.

In the 1997-1998 cruise, two target sites were chosen for mesoscale experiments (magnified areas in figure 2); in these experiments hydrological stations were conducted every 2-3 miles in the areas close to Cape Adare, as well as about 200 km north of the Ross Ice Shelf. These areas are between the continental shelf and slope and have been identified as the site of important meso- to small-scale processes affecting the spreading of antarctic bottom waters formed in the Ross Sea and their interaction with the Circumpolar Deep Water (CDW).

Four mooring lines were deployed in the study area (figure 2). Arrays F (at the edge of the Ross Ice Shelf) and H (near the continental shelf break) are positioned along the axis identified for the diffusion of the Ice Shelf Water (ISW) flowing from below the Ross Ice Shelf towards the slope. Mooring D is located in the Terra Nova Bay polynya, an area of very strong interactions among air, sea and ice with strong influences on ocean processes (such as sea ice production, dense water formation and water ventilation) and on biogeochemical fluxes (gas exchange and vertical convection as a mechanism for carbon transport). Mooring L was deployed during the last cruise, under a cooperative agreement between New Zealand and Italy to preserve and monitor a Site of Special Scientific Interest (SSSI), located in the Terra Nova Bay polynya region south of Tethys Bay over a surface area of 23.5 square kilometers. Each line is equipped with time-series sediment traps, transmissometers, ADCP (Acoustic Doppler Current

Profilers), traditional current meters, and temperature and conductivity sensors.

The research for 1999-2001 involved the participation of scientists working at U.S. (Lamont-Doherty Earth Observatory, Columbia University and the Virginia Institute of Marine Science, College of William and Mary), English (Scott Polar Research Institute, University of Cambridge) and French (Observatoire Océanologique de Banyuls-sur-mer, Université de Paris VI) institutions. These studies included water-mass age and spreading and residence time through the determination of tritium, helium and stable isotopes in water. The 1998-1999 campaign was devoted simply to mooring recovery, maintenance and re-deployment and to XBT and surface drifter launching.