PERGAMON

Deep-Sea Research II 47 (2000) 3249–3272

DEEP-SEA RESEARCH
PART II

www.elsevier.com/locate/dsr2

Microzooplankton herbivory in the Ross Sea, Antarctica

David A. Caron[a,*,1], Mark R. Dennett[a], Darcy J. Lonsdale[b], Dawn M. Moran[a], Ludmilla Shalapyonok[a]

[a]*Department of Biology, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA*
[b]*Marine Sciences Research Center, State University of New York, Stony Brook, NY 11794-5000, USA*

Received 1 June 1999; received in revised form 15 September 1999; accepted 1 October 1999

Abstract

Microplankton abundances and phytoplankton mortality rates were determined at six stations during four cruises spanning three seasons in the Ross Sea polynya, Antarctica (early spring, Oct.–Nov. 1996; mid-late summer, Jan.–Feb. 1997; fall, Apr. 1997; mid-late spring, Nov.–Dec. 1997). Rates of microzooplankton herbivory were measured using a modified dilution technique, as well as by examining the rate of disappearance of phytoplankton (chlorophyll) in samples incubated in the dark (i.e. grazing in the absence of phytoplankton growth). Strong seasonal cycles of phytoplankton and microzooplankton abundance were observed during the study. Microzooplankton abundance varied by more than three orders of magnitude during the four cruises, and was positively correlated with phytoplankton biomass over the entire data set. Nevertheless, microzooplankton grazing was insufficient to impact significantly phytoplankton standing stocks during most of the experiments performed in this perenially cold environment. Only thirteen out of a total of 51 experiments yielded phytoplankton mortality rates that were significantly different from zero. The highest mortality rate observed in this study ($0.26\ d^{-1}$) was modest compared with maximal rates that have been observed in temperate and tropical ecosystems. Results from twenty experiments examining the rate of decrease of phytoplankton biomass during incubations in the dark agreed quite well with the results of the dilution experiments performed at the same time. The range of mortality rates for the dark incubations was -0.09–$0.06\ d^{-1}$, and the average was essentially zero ($-0.01\ d^{-1}$). That is, chlorophyll concentration was virtually unchanged in samples incubated in the dark for 3 d. A number of factors appeared to contribute to the very low rates of microbial herbivory observed, including low water temperature, and the size and

* Corresponding author. Fax: +001-213-740-6720.
E-mail address: dcaron@wrigley.usc.edu (D.A. Caron).
[1] Present address: Department of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0371, USA.

0967-0645/00/$ - see front matter
PII: S0967-0645(00)00067-9

taxonomic composition of the phytoplankton assemblage. Based on our results we conclude that the seasonal, massive phytoplankton blooms observed in the Ross Sea are due, in part, to low rates of removal by microbial herbivores.

1. Introduction

The Ross Sea, Antarctica, is the site of extreme seasonal ranges in rates of primary production and phytoplankton biomass. The annual bloom in this environment is actually a sequential process that follows the retreat of seasonal ice in the polynya, a feature that characterizes and dominates biological processes of the Ross Sea during austral spring and summer (DiTullio and Smith, 1996; Smith et al., 1996; Smith and Gordon, 1997; Smith and Dunbar, 1998). Seasonal maxima of chlorophyll concentrations in excess of $20\,\mu g\,l^{-1}$ have been observed at locations within the polynya. Taxonomic composition of the phytoplankton assemblage in the Ross Sea during this time is somewhat time- and site-specific, but diatoms typically dominate in coastal regions of the sea while the central polynya experiences massive accumulations of the colonial prymnesiophyte *Phaeocystis antarctica* (Smith et al., 1996; Smith and Gordon, 1997).

Biogeographical studies of microbial populations in the Southern Ocean and Antarctic coastal seas during the past two decades have established that nanoplanktonic (2–20 μm) and microplanktonic (20–200 μm) consumers, primarily heterotrophic protists, abound in these perennially cold ecosystems (Garrison et al., 1986; Garrison, 1991; Garrison and Gowing, 1993). These observations, and initial rate measurements of heterotrophic processes in coastal and open waters around Antarctica, have fostered the notion that protozoan assemblages play a major role in the removal of phytoplankton production and biomass in Southern Ocean ecosystems. Indeed, there is a mounting array of data that indicates an active microbial herbivorous fauna in the waters surrounding Antarctica (Taylor and Haberstroh, 1988; Lancelot et al., 1993; Burkill et al., 1995; Froneman and Perissinotto, 1996a, b; Tsuda and Kawaguchi, 1997). Collectively, the results of these studies tend to corroborate conclusions drawn primarily from tropical, subtropical and temperate ecosystems; that phagotrophic protists are major consumers of phytoplankton production and that they play a fundamental role in controlling phytoplankton biomass (Pierce and Turner, 1992; Sherr and Sherr, 1994).

Data regarding the rapid and extensive buildup of phytoplankton biomass in the Ross Sea polynya, however, appear to be in conflict with the general pattern of high rates of herbivory by microbial consumers observed in other oceanic ecosystems. Significant rates of net phytoplankton growth have been documented in the Ross Sea (Smith et al., in press). The massive and highly predictable annual phytoplankton bloom that takes place in the polynya implies that herbivory is not in quantitative balance with the increase in phytoplankton production that occurs in austral spring and summer. It is still possible, however, that phytoplankton growth and grazing

mortality are coupled but temporally offset, with the demise of the bloom mediated by consumers that appear late in the bloom cycle after phytoplankton biomass has increased to high concentration. The validity of these differing scenarios, or alternative explanations, has not been established experimentally.

Studies of microbial herbivory were conducted in the Ross Sea on four cruises spanning three seasons during 1996 and 1997. The purpose of this study was to examine microbial herbivory during the spring, summer and fall in order to document the impact of these consumers on phytoplankton standing stocks and primary production. Phytoplankton mortality was examined using a modified dilution method as well as by examining the loss of phytoplankton biomass during incubations in darkened bottles. Both methods yielded very low rates of removal of phytoplankton biomass. Significant mortality rates were not related to a particular season or to the standing stock of phytoplankton. Massive phytoplankton blooms in the Ross Sea appear to occur because of a complex interaction of factors that include phytoplankton size and composition, as well as a direct effect of extremely low temperature. Our results indirectly support an important role for a detrital food web (microbial loop) in the processing of photosynthetically fixed carbon in the Ross Sea, and perhaps the importance of larger, rarer herbivorous zooplankton.

2. Materials and methods

2.1. Cruise tracks, station locations and water sampling

Sample collection and experimental studies of phytoplankton grazing mortality were conducted during four cruises in the Ross Sea in 1996/97 as a part of the US Joint Global Ocean Flux Study (AESOPS; Antarctic Environment and Southern Ocean Process Study) aboard the R/V *Nathaniel B. Palmer*. The timing of these four cruises provided the opportunity for observations during early austral spring (Oct.–Nov. 1996), mid-late summer (Jan.–Feb. 1997), mid-late autumn (April 1997) and mid-late spring (Nov.–Dec. 1997). Most experiments were conducted at three stations along a single east–west transect line in the Ross Sea (76°30′S) between longitudes 169°E and 178°W. Additional stations were conducted as opportunities arose and included a location in the northern Ross Sea (station "Sei"), one near the Ross Ice Shelf in the vicinity of longitude 176–178°W (station "Emperor"), and two additional stations ("Cooper" and "Merlin") along the transect line and within the longitudinal limits of the three main stations (see Table 1).

Water samples were collected using 20-l Go-Flo bottles deployed in a trace-metal clean rosette from a Kevlar line, or using 10-l Niskin bottles in a standard rosette package when the Go-Flo bottles malfunctioned due to severe cold weather (mostly on the early spring and fall cruises). Water was collected in multiple bottles from the depth of 50% surface irradiance. This depth was chosen to avoid phytoplankton that were light-shocked, and because this irradiance could be reproduced in on-deck

Table 1
Initial parameters and phytoplankton mortality rates at six stations in the Ross Sea during 1996 and 1997[a]

Date	Station Name/No.	Temperature (°C)	Initial total chlorophyll concentration ($\mu g\, l^{-1}$)	Chlorophyll < 5 μm (%)	Chlorophyll < 20 μm (%)	Phytoplankton mortality rate (d^{-1})
Early Austral Spring						
10/20/96	Orca/103	− 1.8	0.14	88	88	0.26[b]
10/23/96	"O"/106	− 1.9	0.13	100	91	NS
10/26/96	Minke/110	− 1.9	0.06	51	59	NS
10/30/96	Orca/111	− 1.8	0.28	92	92	0.23[b]
10/31/96	Emperor/112	− 1.8	0.04	100	100	0.09[c]
11/1/96	Orca/113	− 1.8	0.26	83	88	0.12[b]
11/3/96	Minke/115	− 1.9	0.09	65	74	0.17[b]
11/5/96	Orca/117	− 1.8	0.44	77	93	NS
11/6/96	"O"/120	− 1.8	0.71	77	82	0.15[b]
Mid-late Austral Summer						
1/13/97	Minke/201	0.5	2.0	10	15	NS
1/17/97	"O"/205	− 0.1	5.8	29	28	NS
1/20/97	Orca/208	− 0.2	2.0	45	45	NS
1/22/97	Emperor/209	− 0.4	1.0	40	50	NS
1/25/97	Sei/211	− 0.2	0.70	29	43	NS
1/28/97	Minke/213	0.2	0.65	31	31	0.07[b]
1/31/97	"O"/217	− 0.3	3.6	36	42	NS
2/2/97	Orca/220	− 0.5	0.83	48	72	0.11[b]
Austral Fall						
4/16/97	Orca/304	− 1.8	0.08	75	75	NS
4/18/97	Minke/305	− 1.8	0.04	100	100	NS
4/21/97	Emperor/306	− 1.8	0.05	80	80	NS
4/22/97	Orca/307	− 1.9	0.05	100	100	NS
4/22/97	"O"/310	− 1.8	0.02	100	100	NS
4/26/97	Minke/314	− 1.8	0.03	100	100	NS
4/29/97	Sei/316	− 1.9	0.07	71	86	NS

Mid-late Austral Spring						
11/16/97	Sei/401	− 1.9	0.30	53	97	NS
11/17/97	Orca/402	− 1.8	0.36	53	86	NS
11/19/97	"O"/405	− 1.8	2.4	45	62	NS
11/21/97	Minke/406	− 1.9	0.71	56	62	NS
11/29/97	Orca/416	− 1.7	0.34	59	74	NS
11/30/97	"O"/419	− 1.6	2.6	38	68	NS
12/1/97	Minke/423	− 1.7	1.6	9	15	NS
12/3/97	Orca/425	− 1.8	0.23	74	100	0.10[c]
12/4/97	Merlin/427	− 1.5	4.9	27	66	NS
12/7/97	Cooper/429	− 1.4	10.1	45	45	NS

[a]All data pertain to mortality in seawater filtered through $< 200\ \mu m$ screening. Slopes that are not significantly different from zero are indicated by "NS". Nominal station locations were: Minke = 76°30′S, 169°E; "O" = 76°30′S, 176°E; Orca = 76°30′S, 178°W; Emperor = 78°S, 176°W; Sei = 74°S, 177°E; Cooper = 76°30′S, 170°35′E; Merlin = 76°30′S, 172°35′E.

[b]Mortality rates based on linear regression analysis at 0.01 level.

[c]Mortality rates based on linear regression analysis at 0.05 level.

incubators. Water from the collection bottles was pooled into acid-washed, 50-l polyethylene carboys using silicone tubing, making every effort to minimize bubbling and thereby limit physical damage to delicate plankton. Water handling, experimental preparation, as well as initial and final sampling were performed under subdued light at approximately ambient temperature (0–1°C).

2.2. Phytoplankton biomass and microzooplankton abundance estimates

Measurements of chlorophyll concentration were used to estimate total phytoplankton biomass at each station at the beginning of an experiment. Subsamples of unfiltered seawater were removed from the 50-l carboys and filtered onto GF/F glass fiber filters for chlorophyll measurements. In addition, subsamples of seawater passing 20- and 5-μm inline screens were also collected and analyzed in order to estimate the proportion of small and large phytoplankton. These filtrates were prepared individually (i.e. not sequentially from the same water sample). Chlorophyll was extracted with 100% acetone overnight at -20°C, and samples were read on a Turner Designs Fluorometer.

Microzooplankton abundances were determined at the beginning of each experiment using standard procedures (Stoecker et al., 1994, 1996; Dennett et al., 1999). Subsamples (500 ml) were removed and preserved with Lugols preservative at a final concentration of 10%. Measured aliquots ranging up to 500 ml during periods of low abundance (early spring, fall) of these preserved samples were settled, and counts of major taxonomic categories were performed using an inverted microscope. Dinoflagellates were characterized as phototrophic or heterotrophic based on the presence or absence of chloroplasts in formalin-preserved specimens examined by epifluorescence microscopy.

The relative contributions of diatoms and the colonial haptophyte *P. antarctica* to the phytoplankton assemblages were estimated because these two assemblages dominated the phytoplankton community in most of the experiments. We reasoned that *Phaeocystis* colonies might not be as susceptable to microzooplankton grazing as free-swimming individual *Phaeocystis* cells or other phytoplankton taxa. Diatom abundances were counted in settled samples using an inverted microscope. *Phaeocystis* cells in colonies were determined in two ways. For the early spring cruise (Oct.–Nov. 1996), colonial *Phaeocystis* cells were estimated from colony volume and the number of *Phaeocystis* cells per unit colony volume (counts provided by Sylvie Mathot). For subsequent cruises, colonial *Phaeocystis* cells were approximated from microscopical examination of DAPI-stained samples (25–50 $\mu g\ ml^{-1}$) preserved with 1% formalin and viewed at high magnification using epifluorescence microscopy (Sherr et al., 1993). The colony matrix was dissolved by this treatment, and *Phaeocystis* cells associated with colonies were distinguished based on cell and chloroplast size and shape, and absence of flagella. This method presumably provides a maximal estimate of the number of colonial *Phaeocystis* cells because some similarly shaped cells may not be *P. antarctica*, and some solitary (flagellated) cells may have lost their flagella during sample preservation or staining.

2.3. Dilution experiments

A total of 51 dilution experiments to examine phytoplankton grazing mortality (herbivory) were carried out using a modified dilution technique (Landry et al., 1995b). This method is designed to estimate the rate of mortality (d^{-1}) of the phytoplankton assemblage as a consequence of grazing by planktonic herbivores rather than total mortality (e.g. due to viral lysis, autolysis, sinking, etc.) Our "standard" dilution series consisted of clear polycarbonate bottles (1200 ml) with 20, 40, 60, 80 and 100% seawater filtered by gentle, reverse-flow filtration through 200-μm screening to remove mesozooplankton. Diluent (< 0.2 or 1.2-μm) for all experiments was prepared by gentle gravity filtration using acid-washed, Gelman® cartridge filters, and incubated along with the dilution series. Chlorophyll was virtually undetectable in the 1.2-μm filtrate (cyanobacteria and prochlorophytes were absent from this ecosystem), and therefore it was often used as filtrate in the dilution experiments. We have previously employed cartridge filters for diluent preparation and found that they do not cause detectable increases in dissolved organic carbon or dissolved inorganic nutrients (Caron and Dennett, 1999). Reverse-flow prefiltration (200-μm screening) was performed prior to the preparation of 0.2- or 1.2-μm filtrate for samples that had significant quantities of colonies of the prymnesiophyte *Phaeocystis antarctica* in order to avoid rapid clogging of the cartridges. These latter samples occurred mostly during the mid-late summer cruise (Jan.–Feb. 1997). All dilution treatments were performed in triplicate.

All bottles were enriched with inorganic nutrients and trace metals (10 μM nitrogen as NH_4Cl, 1 μM phosphorus as $Na_2HPO_4 \cdot 7H_2O$, 1 nM iron as FeCl, and 0.1 nM Mg as $MgSO_4 \cdot H_2O$). An additional set of triplicate bottles with < 200 μm seawater without enrichment was prepared (Landry et al., 1995b) and incubated along with control water (diluent) and the dilution series. Incubations for the experiments were performed in on-deck incubators with running seawater to maintain near-ambient water temperature. One layer of neutral grey screening was used to reduce light to approximately 50% of surface irradiance. The length of incubation for most dilution experiments was three days. Incubations were conducted originally for ≈ 1.5 d, but no significant phytoplankton mortality was observed in experiments of that length. Therefore, the incubation time was increased to provide more time for phytoplankton biomass in the various treatments to diverge. Samples were not incubated for more than three days because of the probability of bottle effects with lengthy incubations.

Extremely low grazing mortality rates observed during the study (see Results) prompted us to examine various conditions of our "standard" experimental protocol for the dilution experiments. We performed a number of experiments in conjunction with the protocol described above in which we examined (1) the type of nutrient enrichment in the dilution series, and (2) herbivory in unfiltered seawater (vs. $<$200-μm filtrate). The effect of nutrient enrichment on experimental outcome in the dilution experiments was examined by enriching a replicate set of bottles (i.e. replicating the entire dilution series) with sterile yeast extract at a concentration of 1 mg l^{-1}. Parallel experiments also were performed using natural, unfiltered seawater instead

of seawater prefiltered through 200-μm screening to examine the contribution of mesozooplankton herbivory to total herbivory.

2.4. Phytoplankton mortality in darkened bottles

Experiments were carried out in conjunction with twenty of the dilution experiments during three cruises (mid-late summer, Jan.–Feb. 1997; fall, Apr. 1997; mid-late spring, Nov.–Dec. 1997) to obtain a measure of phytoplankton mortality that was independent from the dilution method. Linear regression analysis for most of the dilution experiments did not yield significant slopes (i.e. mortality rates were indistinguishable from zero; see Results).This finding indicated that either mortality rates were indeed exceedingly low, or that one or more of the assumptions of the method were not valid for this ecosystem (Landry and Hassett, 1982; Landry et al., 1995b). Therefore, parallel experiments were performed to confirm the results obtained with the dilution method.

Subsamples of the same water used for the dilution experiments (prior to filtration through 200-μm screening) were enclosed in three darkened polycarbonate bottles (1200 ml), and incubated along with bottles from the dilution experiments. Chlorophyll concentrations were measured initially, and following three days of incubation (in conjunction with the dilution experiments). Inherent in this approach are the assumptions that phytoplankton growth and photoadaptive changes are negligible in continuous darkness, and that decreases in chlorophyll reflect total phytoplankton mortality (respiratory losses + grazing mortality).

2.5. Hand-collected samples

High abundances of colonies of *P. antarctica* were common at some satations during the mid-late summer cruise (Jan.–Feb. 1997). Experiments were conducted at two of these stations to examine the effect of sample collection and processing on the results of the dilution and "dark bottle" incubations. Collection of seawater in Go-Flo or Niskin bottles and subsequent transfers and handling clearly resulted in some degree of physical disruption to the colonies. In an effort to examine the extent to which this disruption might affect the mortality estimates obtained in our experiments, we collected water that was not subjected to normal bottle collection and processing methods.

Surface waters were sampled from an inflatable boat for these experiments by inverting a wide-mouth, acid-washed polycarbonate carboy in the water, removing the lid, and then opening the spigot to allow water to enter the mouth of the carboy. After filling the carboy completely (to avoid bubbling and sloshing), the lid was replaced underwater, the spigot was closed, and the carboy was returned to the cold room on the ship for processing. Water collected in this manner was used to perform a dilution experiment and a "dark bottle" incubation described above. Seawater for these experiments was gently siphoned from the carboy using a wide-bore silicone tube (to minimize physical agitation). The results of these hand-collected water samples were compared to the results of dilution experiments and "dark bottle"

experiments performed at the same stations using water collected in Go-Flo bottles and processed using our standard handling procedures.

3. Results

3.1. Phytoplankton and microzooplankton abundance:

Chlorophyll concentrations (i.e. phytoplankton biomass) in the Ross Sea ranged over two orders of magnitude (< 0.1 to > 10 μg chlorophyll l^{-1}) on the dates and at the locations examined during this study (Fig. 1A; Table 1). There were significant differences in phytoplankton standing stock among the stations on any cruise, but these differences were minor compared to the overall magnitude and timing of the summer phytoplankton bloom and its demise.

Phytoplankton biomass during early spring (Oct.–Nov. 1996) showed a general west–east gradient in concentration along the main transect line of the study. Station Minke (west end of the transect) did not exceed 0.1 μg chlorophyll l^{-1} on two sampling dates. Stations "O" (approximately the mid-point of the transect) and Orca (eastern end) had higher initial values of 0.13 and 0.14, respectively. Station "O" experienced significant increases in chlorophyll during the cruise (maximum of 0.71) and station Orca to a lesser extent (0.44).

Experiments conducted during the mid-late spring cruise in 1997 began ten Julian days after the last experiment on the 1996 cruise (Table 1). Chlorophyll concentrations during the mid-late spring (Nov.–Dec. 1997) and mid-late summer (Jan.–Feb. 1997) indicated rapid but spatially heterogeneous bloom development along the main transect line. For example, we measured 1.6 μg chlorophyll l^{-1} at station Minke on 12/1/97, but 10.1 μg l^{-1} on 12/7/97 at station Cooper ($< 2^{\circ}$ to the east). All stations showed decreasing chlorophyll concentrations by the end of the mid-late summer cruise. Station Sei, north of the main transect line, and station Emperor south of the line had maximal chlorophyll values of 0.70 and 1.0, respectively, but were sampled too infrequently to note seasonal trends. Consistently low chlorophyll values (0.08 μg l^{-1}) obtained for experiments performed during the April 1997 cruise indicated a very low standing stock of phytoplankton by that time.

Phytoplankton composition at most of the stations was dominated either by diatoms or the colonial prymnesiophyte *P. antarctica* (Dennett et al., submitted). The latter assemblage was highly conspicuous during the latter part of the late spring cruise and throughout the mid-late summer cruise along the main transect. *Phaeocystis* colonies also were common at station Emperor during the mid-late summer cruise. Dominance of this species was reflected in lower percentages of chlorophyll passing through 5-μm and 20-μm screening (Table 1). However, *Phaeocystis* colonies are very fragile and some of these cells may have been extruded through the 20-μm screening. Thus, some of the percentages of total chlorophyll in the < 20-μm filtrates may be overestimates of the phytoplankton biomass less than 20-μm.

The ratio of colonial *P. antarctica* cells to diatoms determined in this study varied from zero (no colonial *Phaeocystis* cells detected) to approximately 5000 for all of the

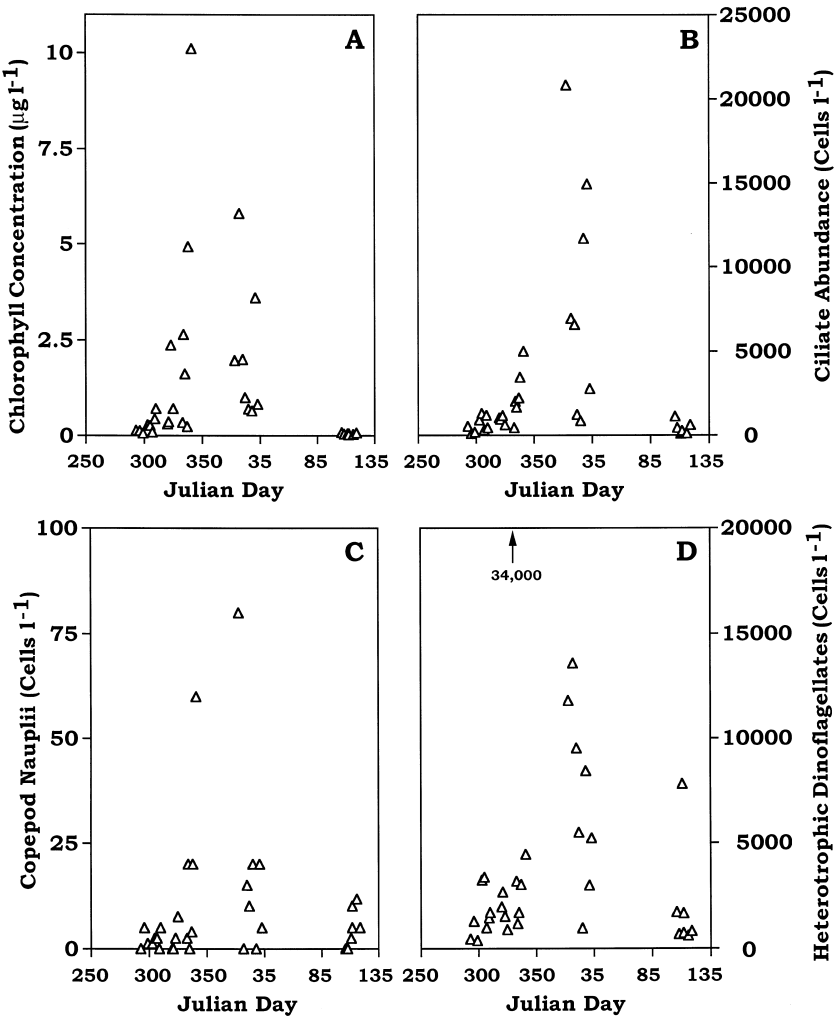

Fig. 1. Chlorophyll (A) and microzooplankton (B–D) concentrations in the Ross Sea at stations where phytoplankton grazing mortality was examined during four cruises during austral spring, summer and fall of 1996 and austral spring of 1997. Data from all four cruises are presented.

dilution experiments performed in this study. The median ratio was 20 (20 *Phaeocystis* cells for every diatom). Direct numerical comparisons do not provide an accurate assessment of dominance of *Phaeocystis* biomass in these samples because the dominant diatom species varied throughout the study. Nevertheless, these results indicate that our experiments included locations and dates that covered a wide range of relative abundances of diatoms and *P. antarctica*.

The microzooplankton assemblages during this study were dominated numerically by heterotrophic dinoflagellates and non-loricate ciliates (Fig. 1). Tintinnid ciliate

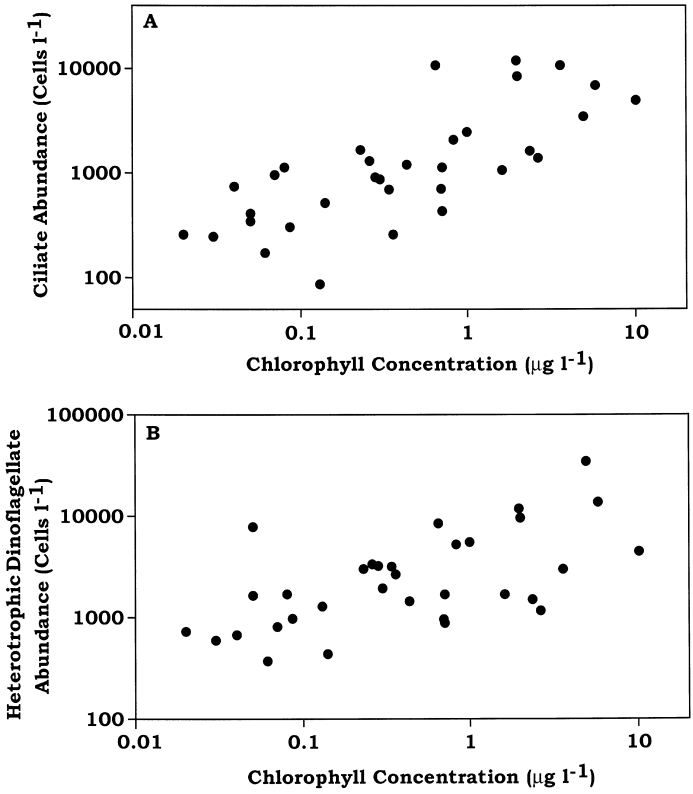

Fig. 2. Relationships between ciliate abundance (A) or heterotrophic dinoflagellate abundance (B) and chlorophyll concentration for four cruises conducted during austral spring, summer and fall of 1996 and austral spring of 1997. Data from all four cruises are presented.

abundances equaled or exceeded the abundances of non-loricate ciliates for only four of the experiments. Heterotrophic dinoflagellates were the most abundant microzooplankton in many of the samples. Heterotrophic dinoflagellates were approximately seven times more abundant than ciliates when averaged over all the dilution experiments (median value was $2.0\times$ as abundant as ciliates). Copepod nauplii never constituted a significant fraction of microzooplankton abundances.

The abundances of both ciliates and heterotrophic dinoflagellates were correlated with chlorophyll concentration ($P < 0.01$) for the entire data set (Fig. 2; correlation coefficients were 0.74 for ciliates and 0.60 for dinoflagellates). This degree of correlation is not surprising given the wide range of chlorophyll values obtained in this study.

3.2. *Phytoplankton mortality in dilution experiments*

A total of 34 dilution experiments were conducted during the four cruises in the Ross Sea using the "standard" protocol described above. The filtrate employed (< 0.2

vs. < 1.2-μm) had no effect on the outcomes of these experiments. Approximately one fourth of these experiments yielded phytoplankton grazing mortality rates that were significantly different than zero when analyzed by linear regression (9 out of 34; Table 1). Mortality rates for these nine experiments ranged up to a maximum of 0.26 d^{-1}. Six of these nine experiments occurred during the early spring cruise (Oct.–Nov. 1996) when chlorophyll concentrations were less than 1.0 $\mu g\, l^{-1}$. Two of the remaining three experiments that yielded measurable phytoplankton mortality occurred during the mid-late summer cruise (Jan.–Feb. 1997) at locations that had some of the lowest chlorophyll concentrations for that cruise (0.65 and 0.83 $\mu g\, l^{-1}$). The last significant rate was observed during late spring (Nov.– Dec. 1997) at the station with the lowest chlorophyll value for that cruise (0.23 $\mu g\, l^{-1}$).

Multiple dilution experiments were performed on thirteen dates during the early spring, summer and fall cruises. Our "standard" dilution protocol was performed, and in addition we altered features of that protocol to determine if it would conspicuously change the grazing mortality rate measurement. These alterations included the use of whole, unfiltered seawater in the dilution series rather than water filtered through 200-μm screening (on eleven dates), and the use of alternative enrichment media (on four dates). The latter was performed to provide more stimulation for phytoplankton growth.

Eleven experiments were performed using unfiltered seawater in the dilution series. We reasoned that *Phaeocystis* colonies present in many of the late spring and summer samples would be retained by the 200-μm filters. Retention would significantly change the phytoplankton assemblage available to microzooplankton grazers in the dilution bottles. In addition, inclusion of mesozooplankton in the unfiltered samples might increase herbivory in the samples. However, filtration through 200-μm screening did not change the chlorophyll concentration as much as expected for samples dominated by large (> 1 mm) *P. antarctica* colonies. For example, during the summer cruise when *Phaeocystis* was abundant, chlorophyll in the < 200-μm filtrate averaged 87% of chlorophyll concentration in unfiltered samples (range of 67–100%). Microscopic examination revealed that the fragile *P. antarctica* colonies were disrupted even by gentle reverse-flow filtration and most passed through the 200-μm screen. Colonies reformed rapidly in these samples (usually within 24 h).

Ten of the eleven dilution experiments performed with unfiltered seawater yielded non-significant slopes (i.e. phytoplankton grazing mortality rates indistinguishable from zero). Six of these samples also yielded non-significant slopes when the experiments were performed using < 200-μm seawater. The remaining four yielded low but significant phytoplankton grazing mortality rates when performed using < 200-μm seawater (rates = 0.07–0.23 d^{-1}). The one dilution experiment using unfiltered seawater that yielded a significant mortality rate (0.18 d^{-1}) showed no significant rate when < 200-μm seawater was employed.

Four experiments were performed in which yeast extract (three experiments at 1.0 $mg\, l^{-1}$, and one experiment at 10 $mg\, l^{-1}$) was used as the enrichment medium instead of inorganic nutrients and trace metals. Yeast extract is a complex mixture of organic substrates and inorganic nutrients that should provide substrate for heterotrophic bacteria as well as inorganic nutrients for phytoplankton. We speculated that

stimulation of the bacterial assemblage might have some effect on phytoplankton growth and grazing. The "standard" dilution protocol for all four of these experiments yielded phytoplankton grazing mortality rates that did not significantly differ from zero, and all four of the dilution series enriched with yeast extract yielded the same result.

Phytoplankton growth rates determined from the dilution experiments were affected by dramatic changes in chlorophyll cell^{-1} during the incubations. Chlorophyll content decreased during the incubations, particularly for *P. antarctica*, presumably as a consequence of light quality in our incubators. This decrease in cell^{-1} chlorophyll concentration has been observed previously (W.O. Smith, Jr., personal communication). Irradiance in the on-deck incubators was diminished to match irradiance at the sampling depth, but we could not match spectral quality of the light. Phytoplankton production was apparently not adversely affected during these incubations (based on primary productivity measurements by other JGOFS investigators), but the growth rate estimates based on changes in chlorophyll during the dilution incubations were negative for many of the samples on all cruises, and these growth rates are not presented. Grazing mortality rates, however, should be unaffected by changes in chlorophyll cell^{-1} assuming that photoadaptation was the same for phytoplankton in all bottles of the dilution series (i.e. no significant differences in "self-shading"). This assumption is reasonable given the moderate phytoplankton biomass values for the samples.

3.3. Phytoplankton mortality in darkened bottles and hand-collected samples

We conducted experimental studies along with the dilution experiments in order to try to determine if the potential artifact described above was important for our studies. We conducted experiments in which aliquots of unfiltered, gently collected seawater were placed into blackened bottles (to prevent light from entering the bottles) and incubated for three days. Subsamples were collected at the beginning and at the end of the incubations and analyzed for changes in the concentration of chlorophyll over the course of the incubations. We reasoned that we should observe significant decreases in chlorophyll due to the cessation of phytoplankton growth but continued activity of herbivorous zooplankton (assuming that grazing activity did not completely cease in the dark). Surprisingly, we observed virtually no decreases in chlorophyll in any of these experiments during three day incubations in the dark (Table 2). Average mortality rates for three cruises on which these experiments were performed were 0.01, 0.02 and 0.01 d^{-1} for mid-late summer, fall and mid-late spring, respectively (overall range $= -0.04$ to 0.10 d^{-1}).

Two experiments were conducted to examine the effects of sample handling on phytoplankton mortality rate. We reasoned that collection of seawater by rosette sampler might cause significant damage to delicate microplankton, and thus result in low phytoplankton mortality rates. Water samples were collected for these experiments by rosette sampler and by hand (see Methods and Materials). Grazing mortality rates were determined by the dilution technique and by the incubation of unfiltered seawater in the dark.

Table 2
Phytoplankton mortality rates based on changes in chlorophyll concentration during three-day incubations in the dark[a]

Date	Station name/no.	Mortality (d^{-1})
Mid-late Austral Summer		
1/28/97	Minke/213	− 0.04
1/31/97	"O"/217	0.08
2/2/97	Orca/220	0.00
		Average = 0.01
Austral Fall		
4/16/97	Orca/304	0.06
4/18/97	Minke/305	− 0.03
4/21/97	Emperor/306	0.02
4/22/97	Orca/307	0.00
4/22/97	"O"/310	0.00
4/26/97	Minke/314	0.10
4/29/97	Sei/316	− 0.03
		Average = 0.02
Mid-late Austral Spring		
11/16/97	Sei/401	0.00
11/17/97	Orca/402	0.08
11/19/97	"O"/405	− 0.06
11/21/97	Minke/406	0.00
11/29/97	Orca/416	0.01
11/30/97	"O"/419	− 0.02
12/1/97	Minke/423	− 0.02
12/3/97	Orca/425	− 0.03
12/4/97	Merlin/427	0.05
12/7/97	Cooper/429	0.05
		Average = 0.01

[a]All rates are expressed as mortality. Negative numbers indicate an increase in chlorophyll during the incubation period.

The method of water collection did not indicate a major effect of sample handling on the resulting phytoplankton mortality rates (Table 3). Slight differences in rates were obtained for the different methods of water collection and experimental protocol but, overall, rates were low. The highest rate obtained for all permutations was 0.15 d^{-1} for a hand-collected sample.

3.4. Phytoplankton mortality and temperature

Overall, our phytoplankton grazing mortality rates from the Ross Sea were exceedingly low during all cruises relative to other marine studies in other locales using similar methodology (Tables 1–3). We examined the effect of temperature on mortality rates as one obvious potential explanation for these low rates using an array of

Table 3
Comparison of phytoplankton mortality rates in hand-collected samples and samples obtained in Go-Flo bottles using a rosette sampler during late austral summer 1997 in the Ross Sea[a]

Date	Rosette-collected		Hand-collected	
	Based on dilution	Based on dark incubations	Based on dilution	Based on dark incubations
1/31/97	NS[b]	0.08	0.06	0.02
2/2/97	0.11	0.00	0.08	0.15

[a]Mortality rates were determined using the dilution protocol and by the disappearance of chlorophyll in samples incubated in the dark (all rates are d^{-1}).
[b]Indicates a non-significant regression for a dilution experiment.

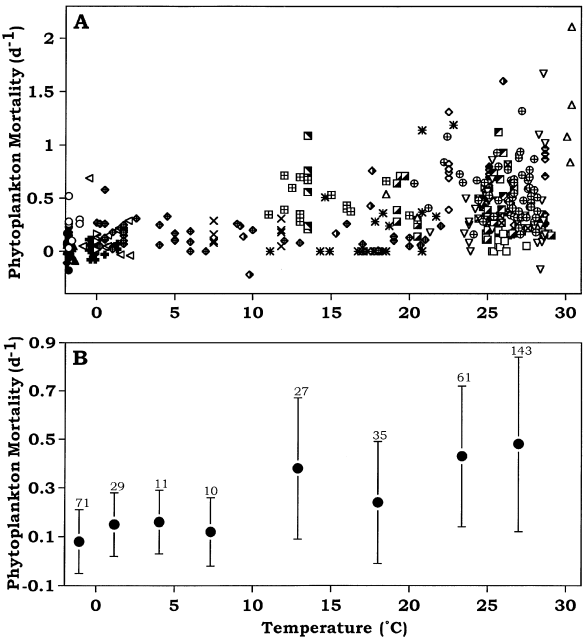

Fig. 3. Summary of phytoplankton grazing mortality rates (d^{-1}) from a variety of marine ecosystems determined using the dilution technique as a function of water temperature at the time and location of the experiments. Scatter diagram of individual grazing mortality rates as a function of temperature (A), and the same data (B) binned by temperature interval (intervals were $\leqslant 0°$, $> 0° \leqslant 2°$, $> 2° \leqslant 6°$, $> 6° \leqslant 10°$, $> 10° \leqslant 15°$, $> 15° \leqslant 20°$, $> 20° \leqslant 25°$ and $> 25°$C). Mean grazing mortality rates (d^{-1}) are indicated by filled circles and plotted at the mean experimental temperature of the experiments. Error bars are ± 1 standard deviation, and numbers above each bar indicate the number of experiments on which the averages are based. Legend for panel A: (□) Ayukai (1996); (◇) Ayukai & Miller (1998); (○) Burkill et al. (1995); (△) Dagg (1995); (⊞) Froneman & Perissinotto (1996a); (⊕) Froneman & Perissinotto (1996b); (⊕) Landry et al. (1998); (▽) Landry et al. (1995); (◪) Lessard & Murrell (1998); (◈) McManus & Ederington Cantrell (1992); (✱) Murrell & Hollibaugh (1998); (▷) Paranjape (1987); (◩) Reckermann & Velduis (1997); (✕) Strom et al. (1991); (◁) Tsuda & Kawaguchi (1997); (◨) Verity et al. (1993); (◈) Verity et al. (1996); (⊠) Caron & Dennett (1999); (●) This study; early spring; (✚) This study; mid-late summer; (◆) This study; fall; (▲) This stidy; late spring.

published studies. Experimental investigations were included that employed the dilution technique for a wide variety of marine ecosystems, and in which experimental temperatures were reported (Fig. 3). There was considerable scatter in the data of this plot (Fig. 3A), as would be expected because mortality rates at any particular temperature are affected by other factors including prey abundance, size, quality and microzooplankton abundance. Undetectable mortality rates (i.e. the slope of the regression between apparent phytoplankton growth rate and relative grazing pressure was not significant) were depicted as no grazing mortality. Nevertheless, there was a trend for high mortality rates (d^{-1}) to occur only at warmer temperatures. In fact, only experiments conducted at temperatures $\geqslant 10^{\circ}C$ resulted in mortality rates greater than $0.35\ d^{-1}$ for these studies from a wide array of marine environments (Fig. 3A).

The relationship between grazing mortality rate and temperature for these studies was further examined by binning the rate data according to experimental temperature ($\leqslant 0^{\circ}$, $>0^{\circ} \leqslant 2^{\circ}$, $>2^{\circ} \leqslant 6^{\circ}$, $>6^{\circ} \leqslant 10^{\circ}$, $>10^{\circ} \leqslant 15^{\circ}$, $>15^{\circ} \leqslant 20^{\circ}$, $>20^{\circ} \leqslant 25^{\circ}$ and $>25^{\circ}C$). A comparison of means and variances associated with these grouped data indicated a significant effect of temperature (Fig. 3B). Mean grazing mortality rate for experiments conducted at temperatures $\leqslant 0^{\circ}C$ were significantly different from mean rates in all temperature intervals $>10^{\circ}C$ ($p < 0.001$),. Similarly, mean rates for experiments conducted at temperatures $>0 \leqslant 2^{\circ}C$ were significantly different from mean rates at temperatures $>10^{\circ}C$ ($p < 0.001$) except for the 15–20° interval. The only remaining significant difference occurred between the rates obtained for the temperature intervals 15–20° and $>25^{\circ}$.

4. Discussion

We observed extremely low rates of phytoplankton grazing mortality using the dilution technique in this study. Low rates of phytoplankton mortality are not novel, but this study was unusual in that nearly all measurements collected during four cruises were extremely low, if significantly different from zero. These rates were not a result of low microzooplankton abundance during the study (Figs. 1 and 2). Overall, abundances of microzooplankton (dominated by ciliates, heterotrophic dinoflagellates) during this study were similar to and in some cases greater than microzooplankton abundances observed using comparable methodology during the North Atlantic Bloom, Equatorial Pacific, and Arabian Sea JGOFS studies (Stoecker et al., 1994, 1996; Dennett et al., 1999; Garrison et al., 1998). Therefore, the very low rates of phytoplankton mortality that we observed cannot be reconciled by the relative absence of potential consumers of phytoplankton biomass.

Low rates of herbivory in the Ross Sea also cannot be explained by an overabundance of phytoplankton prey (at least not in most samples). High phytoplankton biomass can result in reductions in clearance rates of microzooplankton. This situation was clearly not an issue during most of our experiments where chlorophyll concentrations were moderate to very low. It is possible that phytoplankton biomass did suppress microzooplankton clearance rates during the spring–summer bloom

period, but 2- and 3-point regressions of our dilution data (Gallegos, 1989) did not change the outcome of any of the experiments during those periods. It is interesting, however, that the experiments that yielded significant (albeit low) rates of phytoplankton grazing mortality in this study occurred typically at low-to-intermediate concentrations of chlorophyll (Table 1).

Initially, our low phytoplankton grazing mortality rates led us to suspect that one or more of the assumptions of the dilution method might have been violated, or perhaps sample handling caused damage to delicate phytoplankton or microbial consumers. However, this latter situation seemed unlikely as an explanation for the low rates that we observed. Non-toxic, acid-washed materials were employed in the experiments, and bubbling of water samples during transfer (which can damage some protists) was minimized. Preparation of the dilution series and incubation of all samples were performed at ambient temperature and under subdued light to avoid heat or light shock to the plankton assemblage. In addition, an extremely gentle method of sample collection (i.e. hand-collection) in the present study and minimal physical disturbance during experimental setup still resulted in phytoplankton mortality rates that were consistently low (Table 3). Our routine experimental protocol used in the present study worked well in a recent study in the Arabian Sea (Caron and Dennett, 1999). Yet the lowest phytoplankton mortality rates observed in the latter study were approximately equivalent to the highest rate observed in the present study ($0.26\ d^{-1}$).

Mass mortality of microbial herbivores also could explain the low rates of phytoplankton grazing mortality that we observed, but we could find no evidence for this situation. Microzooplankton growth rates determined for several experiments during austral summer in the present study varied geographically within the Ross Sea (range of -0.5–$1.0\ d^{-1}$) but the overall average was approximately $0.1\ d^{-1}$ (Lonsdale et al., 2000). These results do not support the hypothesis that usually high mortality of microbial consumers occurred during our experiments.

Non-significant regressions in our dilution experiments also could have been caused by failure to meet one or more of the assumptions of the method (Landry and Hassett, 1982; Landry et al., 1995b). Major assumptions for this technique are that phytoplankton growth is adequately described by an exponential model, growth is independent of the dilution (i.e. the same in all bottles), and that grazing mortality varies directly with the degree of dilution (i.e. herbivore clearance rates are maximal and constant, and herbivore impact is inversely related to the degree of dilution).

Failure to meet the first of these assumptions could have resulted in slight underestimations of mortality rates in the present study, but it would not have explained consistently negligible mortality. Similarly, it is unlikely that phytoplankton growth was significantly different among the bottles. The modified dilution technique (Landry et al., 1995b) employs nutrient supplementation to all bottles in order to avoid nutrient limitation of the phytoplankton, and thus eliminates dependence of phytoplankton growth on the presence of microzooplankton. Therefore, it is unlikely that differences in phytoplankton growth rates among the bottles of the dilution series could explain our results. Even supplementation of our dilution experiments with

a complex mixture of inorganic and organic compounds (yeast extract) did not have an effect on the experimental outcomes.

The presence of the *Phaeocystis* colonies along the main cruise track and at station Emperor in the Ross Sea during the mid-late spring and mid-late summer cruises could have violated the last assumption (mortality varies directly with the degree of dilution). We noted that a small proportion of these prymnesiophyte colonies had microzooplankton associated with them, in many cases actually inside the hollow, gelatinous colonies. Dilution of a seawater sample would not have resulted in the sequential reduction in abundance of these consumers because they would simply be transferred to the diluted bottles along with the colonies. The overall effect of violating this assumption of the method would be to underestimate mortality rates because dilution of the phytoplankton samples would not result in reduction of grazing mortality (that is, phytoplankton growth and mortality would be the same in all bottles if all the grazers are associated with and transferred with *Phaeocystis* colonies).

Dilution of the samples, however, should have resulted in dilution of herbivores not directly associated with the colonies, so one might expect reduced (but not necessarily zero) mortality rates to result if grazing by microzooplankton associated with *Phaeocystis* colonies constituted a significant fraction of total microzooplankton herbivory. However, "infected" colonies never dominated in any of our experiments (based on microscopical examination of live material), and we noted that filtration through 200 μm netting to exclude mesozooplankton resulted in the breakage of many of the *Phaeocystis* colonies. This disruption of the colony integrity presumably freed the microzooplankton from the colonies and allowed their dilution during the preparation of the dilution series.

Nevertheless, the very low phytoplankton grazing mortality rates that we observed using the dilution method prompted us to examine the validity of the rates that we obtained. We therefore conducted two types of experiments to determine whether the dilution method was providing an accurate appraisal of microzooplankton grazing activities in our Ross Sea samples.

Phytoplankton mortality rates obtained by following changes in chlorophyll concentration in samples incubated in the dark substantiated our findings based on the dilution method (Table 2). Changes in chlorophyll during three day incubations in the dark during 20 experiments carried out during three of the four cruises were often within the analytical error of the method. That is, chlorophyll concentration (and presumably phytoplankton biomass) were essentially unchanged after three days. Significant photoadaptation is unlikely in continuous darkness, so these results indicate exceedingly low phytoplankton mortality in these experiments.

4.1. Temperature and microzooplankton herbivory

Water temperatures in the Ross Sea polynya were exceedingly cold throughout this study. Only the experiments that were conducted during austral summer had temperatures in excess of -1.4°C, and temperatures in the latter experiments never exceeded $+0.5^{\circ}$C (Table 1). Low temperature appeared to be a significant factor limiting the

rate of microzooplankton herbivory in this antarctic ecosystem. This conclusion is not without precedent, although this general relationship has not been reported previously.

Tsuda and Kawaguchi (1997) conducted dilution experiments in the Indo-Pacific and Atlantic sectors of the Southern Ocean where temperatures ranged from approximately -1 to 2°C. Most phytoplankton grazing mortality rates in that study were low or moderate ($\leqslant 0.29\ d^{-1}$) with one exception ($0.69\ d^{-1}$). They observed balanced phytoplankton growth and grazing in a number of samples, but phytoplankton growth exceeded grazing at several stations. Similarly, Paranjape (1987) also determined moderate phytoplankton mortality rates for experiments performed at 0°C in Baffin Bay ($0.01–0.17\ d^{-1}$).

A positive correlation between temperature and microzooplankton herbivory ($\mu g\ C\ consumed\ l^{-1}\ d^{-1}$) has been previously noted in the Bellingshausen Sea (Burkill et al., 1995). Likewise, Froneman et al. (1996) measured very low rates of phytoplankton mortality using a dilution technique at the ice edge zone of the Lazorev Sea near Antarctica (temperatures were not reported). Archer et al. (1996) reported rates of herbivory by dominant species of heterotrophic dinoflagellates in coastal waters of East Antarctica. In-situ temperatures in that study were comparable to those of this study (-0.4 to -1.4°C). The authors used ^{14}C to estimate the grazing rates of nine dinoflagellates, and then calculated their impact on the phytoplankton community. Their calculations indicated that these species consumed modest percentages of the standing stock of autotrophs each day (0.51–13.79%). Cell-specific clearance rates for heterotrophic dinoflagellates were approximately one order of magnitude lower than values obtained in other studies. Both low temperature and phytoplankton community composition were suggested as possible factors affecting these rates.

Our analysis of a large set of published phytoplankton grazing mortality data (and our own results) obtained using the dilution method indicated that, on average, dilution experiments conducted at temperatures $\leqslant 2$°C yielded significantly lower phytoplankton grazing mortality rates than for experiments conducted at environmental temperatures > 10°C (Fig. 3B). While experiments conducted at temperatures > 10°C yielded a wide range of mortality rates (from undetectable up to $> 2\ d^{-1}$), grazing mortality rates $> 0.4\ d^{-1}$ were very rare at low temperatures. We speculate that low temperature had a direct effect on $cell^{-1}$ rates of ingestion or digestion by the microzooplankton. It is possible, however, that the effect of temperature was mediated through some other aspect of trophic coupling that was affected by temperature (e.g. phytoplankton size or nutritional quality; see below).

4.2. Phytoplankton community composition and microzooplankton herbivory

Chlorophyll concentration ranged over two orders of magnitude in the present study, and therefore was not, by itself, an explanation for our low rates of phytoplankton mortality. Phytoplankton community composition, however, has been implicated as an important factor affecting microzooplankton feeding activities. Diatoms and the colonial prymnesiophyte *P. antarctica* dominated most of the samples examined in the

present study (Dennett et al., submitted). These populations may both be capable of deterring microzooplankton predation pressure to some extent.

Numerous studies have examined grazing on species of *Phaeocystis* (Weisse et al., 1994). In general, colony formation and possible physiological condition of the cells can reduce grazing on these species by microzooplankton. Admiraal and Venekamp (1986) noted high densities of tintinnids and high rates of grazing on single cells of *Phaeocystis pouchetti* in the Dutch Wadden Sea and North Sea. Similarly, Weisse and Scheffel-Möser (1990) examined growth and grazing of single cells of *Phaeocystis* sp. in the North Sea using a dilution approach (temperature $\approx$ 8–10°C). They observed relatively close balance between growth and grazing losses. We might have expected significant grazing losses during bloom initiation (when single cells of *Phaeocystis* typically dominate) if we extrapolate their results to our study. Interestingly, six of the nine dilution experiments that yielded significant mortality rates in our study were observed during early austral spring, when single cells of *P. antarctica* would be expected to dominate.

Conversely, the formation of colonies of *P. antarctica* may deter grazing by many microzooplankton. Low rates of phytoplankton grazing mortality have been observed during *Phaeocystis* blooms in the North Sea (Brussaard et al., 1996). These authors reported that cell lysis of *Phaeocystis*, rather than grazer-mediated mortality, as a major factor in the decline of the bloom.

Species of *Phaeocystis* have been reported as being unpalatable to mesozooplankton species, particularly in the colonial stage. This effect may be related to the physiological condition of the colonies (Estep et al., 1990). It has been suggested that selective grazing of mesozooplankton on microzooplankton in such a scenario might act to reduce the grazing pressure of protists on *Phaeocystis*, creating a "cascade effect" that enhances the ability of *Phaeocystis* to bloom (Hansen et al., 1993). This scenario is consistent with our findings in the central Ross Sea polynya were *Phaeocystis* dominated during austral summer. A companion paper (Lonsdale et al., 2000) reports on microzooplankton consumption by mesozooplankton. However, we did not observe particularly low abundances of microzooplankton that might be expected if this assemblage were under heavy grazing pressure. An alternative hypothesis that links bloom initiation by *P. antarctica* to its growth potential at low light intensity may contribute to the success of this species in the Ross Sea (Moison and Mitchell, 1999).

The presence *P. antarctica* may be a factor in explaining the low rates of phytoplankton grazing mortality that we observed in the Ross Sea, but it cannot be the only factor. We found no relationship between the numerical dominance of diatom cells and whether or not a significant mortality rate was obtained in a dilution experiment. Experiments with significant mortality rates spanned almost the entire range of diatom: *Phaeocystis* ratios (0.9–5000). That is, insignificant slopes in the dilution experiments could not be explained simply by a dominance of a presumably "poor quality" food (*P. antarctica*).

It is probable that some diatoms also may deter microzooplankton grazing to some extent. Many of these species are too large or too irregular in shape for many herbivorous ciliates in the plankton, and heterotrophic dinoflagellates appear to be

the major microzooplanktonic consumers of these phytoplankton (Jacobson and Anderson, 1986; Strom and Buskey, 1993; Verity et al., 1993). If rates of ingestion by heterotrophic dinoflagellates are depressed relative to rates at warmer temperatures (Archer et al., 1996), or if the diatoms are sufficiently large, then phytoplankton grazing mortality may be concomitantly reduced.

5. Conclusions

We observed low overall phytoplankton mortality rates in the Ross Sea polynya during the US JGOFS process study. These low rates are in accordance with other experimental and observational results in these antarctic waters. The enormous buildup of phytoplankton biomass in the Ross Sea polynya is evidence that phytoplankton growth exceeds mortality for a significant period of austral spring and summer. Therefore, one might expect that daily losses of the phytoplankton assemblage due to grazing mortality would constitute a relatively small percentage of the total standing stock of phytoplankton for much of the bloom period. One also might expect that the decline of the bloom would be marked by increased grazer mortality, but we observed no clear evidence of this situation during late austral summer 1997. On the other hand, DiTullio (1998) has reported evidence for a major sinking event following a *Phaeocystis* bloom in the Ross Sea, and Asper et al. (1996) have noted evidence for this vertical flux from sediment trap collections. These observations imply that much of the bloom is not consumed at the time of its production, but is removed by aggregation and sinking. Our results are consistent with that speculation.

We conclude that much of the organic material produced during austral spring and summer in the Ross Sea is not consumed immediately by microzooplankton, although direct consumption of phytoplankton biomass by meso- and macrozooplankton cannot be ruled out. In particular, the potential importance of large, microphagous zooplankton in Antarctic ecosystems has been proposed (Le Fèvre et al., 1998). The contribution of meso- and macrozooplankton to phytoplankton mortality cannot be assayed adequately using the dilution method.

Based on our findings of low phytoplankton mortality presented here, and our observations of high abundances of bacterivorous protists (choanoflagellates) during late austral summer (Caron et al., 1999), we speculate that scenescence and/or lysis of the algal assemblage and subsequent microbial degradation appear to play an important role in the mobilization of algal material through the pelagic food web of the Ross Sea. If true, bacterivory by nano/microplanktonic consumers may constitute an important route for the flow of energy in pelagic waters of this ecosystem.

Acknowledgements

We gratefully acknowledge the assistance of the captain and crew of the R/V *Nathaniel B. Palmer*, the US JGOFS Office and Antarctic Support Associates for logistical and cruise support. We are grateful to Dr. Sylvie Mathot who supplied

information on heterotrophic and phototrophic dinoflagellates and colonial *P. antarctica* cell abundances for the Oct.–Nov. 1996 cruise. We also thank Dr. Ee Lin Lim, Geraldine Miceli and Michael Doall for technical assistance during the cruises. This study was supported by National Science Foundation grant OCE-9633703 and OCE-9634241. Contribution No. 10278 from Woods Hole Oceanographic Institution. US JGOFS Contribution No. 578.

References

Admiraal, W., Venekamp, L.A.H., 1986. Significance of tintinnid grazing during blooms of *Phaeocystis pouchetii* (Haptophyceae) in dutch coastal waters. Netherlands Journal of Sea Research 20, 61–66.

Archer, S.D., Leakey, R.J.G., Burkill, P.H., Sleigh, M.A., 1996. Microbial dynamics in coastal waters of east Antarctica: herbivory by heterotrophic dinoflagellates. Marine Ecology Progress Series 139, 239–255.

Asper, V.L., Diercks, A.-R., Tuel, M.D., 1996. Fluxes of carbon and total mass in the Ross Sea polynya during the early austrual spring, 1994. EOS 76, 137 (Abstract).

Ayukai, T., 1996. Possible limitation of the dilution technique for estimating growth and grazing mortality rates of picoplanktonic cyanobacteria in oligotrophic tropical waters. Journal of Experimental Marine Biology and Ecology 198, 101–111.

Ayukai, T., Miller, D., 1998. Phytoplankton biomass, production and grazing mortality in Exmouth Gulf, a shallow embayment on the arid, tropical coast of Western Australia. Journal of Experimental Marine Biology and Ecology 225, 239–251.

Brussaard, C.P.D., Gast, G.J., van Duyl, F.C., Riegman, R., 1996. Impact of phytoplankton bloom magnitude on a pelagic microbial food web. Marine Ecology Progress Series 144, 211–221.

Burkill, P.H., Edwards, E.S., Sleigh, M.A., 1995. Microzooplankton and their role in controlling phytoplankton growth in the marginal ice zone of the Bellingshausen Sea. Deep-Sea Research 42, 1277–1290.

Caron, D.A., Dennett, M.R., 1999. Phytoplankton growth and mortality during the 1995 Northeast Monsoon and Spring Intermonsoon in the Arabian Sea. Deep-Sea Research II 46, 1665–1690.

Caron, D.A., Lonsdale, D.J., Dennett, M.R., 1999. Bacterivory and herbivory play key roles in fate of Ross Sea production. Antarctic Journal of the United States 32, 81–83.

Dagg, M.J., 1995. Ingestion of phytoplankton by the micro- and mesozooplankton communities in a productive subtropical estuary. Journal of Plankton Research 17, 845–857.

Dennett, M.R., Caron, D.A., Mursov, S., Polikarpov, I.G., Gavrilova, N.A., Georgieva, L.V., Kuzmenko, L.V., 1999. Abundance and biomass of nano- and microplankton during the 1995 Northeast Monsoon and Spring Intermonsoon in the Arabian Sea. Deep-Sea Research II 46, 1691–1771.

Dennett, M.R., Mathot, S., Caron, D.A., Smith, W.O., Jr., Lonsdale, L. Abundance and distribution of phototrophic and heterotrophic nano- and microplankton in the southern Ross Sea. Deep-Sea Research II, submitted.

DiTullio, G.R., 1998. Export of *Phaeocystis antarctica* to deep water and sediments of the Ross Sea. Ocean Sciences Meeting, American Geophysical Union, Vol. 78, February 9–13 (Abstr.).

DiTullio, G.R., Smith Jr., W.O., 1996. Spatial patterns in phytoplankton biomass and pigment distributions in the Ross Sea. Journal of Geophysical Research 101, 18467–18478.

Estep, K.W., Nejstgaard, J.C., Skjoldal, H.R., Rey, F., 1990. Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. Marine Ecology Progress Series 67, 235–249.

Froneman, P., Perissinotto, R., 1996a. Microzooplankton grazing and protozooplankton community structure in the South Atlantic and in the Atlantic sector of the Southern Ocean. Deep-Sea Research 43, 703–721.

Froneman, P., Perissinotto, R., 1996b. Microzooplankton grazing in the Southern Ocean: implications for the carbon cycle. P.S.Z.N.I.: Marine Ecology 17, 99–115.

Froneman, P., Perissinotto, R., 1996c. Structure and grazing of the microzooplankton communities of the Subtropical convergence and a warm-core eddy in the Atlantic sector of the Southern Ocean. Marine Ecology Progress Series 135, 237–245.

Froneman, P., Perissinotto, R., McQuaid, C.D., 1996. Dynamics of microplankton communities at the ice-edge zone of the Lazarev Sea during a summer drogue study. Journal of Plankton Research 18, 1455–1470.

Gallegos, C.L., 1989. Microzooplankton grazing on phytoplankton in the Rhode River, Maryland: non linear feeding kinetics. Marine Ecology Progress Series 57, 23–33.

Garrison, D.L., 1991. An overview of the abundance and role of protozooplankton in Antarctic waters. Journal of Marine Systems 2, 317–331.

Garrison, D.L., Gowing, M.M., 1993. Protozooplankton. In: Friedmann, E.I. (Ed.), Antarctic Microbiology. Wiley-Liss, New York, pp. 123–166.

Garrison, D.L., Gowing, M.M., Hughes, M.P., 1998. Nano- and microplankton assemblages in the northern Arabian Sea during the Southwest Monsoon, August–September, 1995: a US-JGOFS study. Deep-Sea Research II 45, 2269–2299.

Garrison, D.L., Sullivan, C.W., Ackley, S.F., 1986. Sea ice microbial communities in Antarctica. Bioscience 36, 243–250.

Hansen, F.C., Reckermann, M., Klein Breteler, W.C.M., Riegman, R., 1993. *Phaeocystis* blooming enhanced by copepod predation on protozoa: evidence from incubation experiments. Marine Ecology Progress Series 102, 51–57.

Jacobson, D.M., Anderson, D.M., 1986. Thecate heterotrophic dinoflagellates: feeding behaviour and mechanisms. Journal of Phycology 22, 249–258.

Lancelot, C., Mathot, S., Becquevort, S., Dandois, J.-M., Billen, G., 1993. Carbon and nitrogen cycling through the microbial network of the marginal ice zone of the Southern Ocean with particular emphasis on the northwestern Weddell Sea. In: Caschetto, S. (Ed.), Belgian Scientific Research Programme on the Antarctic. Scientific Results of Phase Two (10/1988–05/1992), Vol. I. Plankton Ecology and Marine Biogeochemistry. Belgian Science Policy Office, Brussels, pp. 1–90.

Landry, J.R., Brown, S.L., Campbell, L., Constantinou, J., Liu, H., 1998. Spatial patterns in phytoplankton growth and microzooplankton grazing in the Arabian Sea during monsoon forcing. Deep-Sea Research 45, 2353–2368.

Landry, M.R., Constantinou, J., Kirshtein, J., 1995a. Microzooplankton grazing in the central equatorial Pacific during February and August, 1992. Deep-Sea Research II 42, 657–671.

Landry, M.R., Hassett, R.P., 1982. Estimating the grazing impact of marine micro-zooplankton. Marine Biology 67, 283–288.

Landry, M.R., Kirshtein, J., Constantinou, J., 1995b. A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental test in the central equatorial Pacific. Marine Ecology Progress Series 120, 53–63.

Le Fèvre, J., Legendre, L., Rivkin, R.B., 1998. Fluxes of biogenic carbon in the Southern Ocean: roles of large microphagous zooplankton. Journal of Marine Systems 17, 325–345.

Lessard, E.J., Murrell, M.C., 1998. Microzooplankton herbivory and phytoplankton growth in the northwestern Sargasso Sea. Aquatic Microbial Ecology 16, 173–188.

Lonsdale, D.J., Caron, D.A., Dennett, M.R., Shaffner, R., 2000. Predation by *Oithona* sp. on protozooplankton in the Ross Sea, Antarctica. Deep-Sea Research II 47, 3273–3283.

McManus, G.B., Ederington-Cantrell, M.C., 1992. Phytoplankton pigments and growth rates, and microzooplankton grazing in a large temperate estuary. Marine Ecology Progress Series 87, 77–85.

Moison, T.A., Mitchell, B.G., 1999. Photophysiological acclimation of *Phaeocystis antarctica* Karsten under light limitation. Limnology and Oceanography 44, 247–258.

Murrell, M.C., Hollibaugh, J.T., 1998. Microzooplankton grazing in northern San Francisco Bay measured by the dilution method. Aquatic Microbial Ecology 15, 53–63.

Paranjape, M.A., 1987. Grazing by microzooplankton in the eastern Canadian arctic in summer 1983. Marine Ecology Progress Series 40, 239–246.

Pierce, R.W., Turner, J.T., 1992. Ecology of planktonic ciliates in marine food webs. Reviews in Aquatic Science 6, 139–181.

Reckermann, M., Veldhuis, M.J.W., 1997. Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE Monsoon 1993. Aquatic Microbial Ecology 12, 263–273.

Sherr, E.B., Caron, D.A., Sherr, B.F., 1993. Staining of heterotrophic protists for visualization via epifluorescence microscopy. In: Kemp, P., Sherr, B., Sherr, E., Cole, J. (Eds.), Handbook of Methods in Aquatic Microbial Ecology. Lewis Publishers, Boca Raton, pp. 213–227.

Sherr, E.B., Sherr, B.F., 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microbial Ecology 28, 223–235.

Smith Jr., W.O., Dunbar, R.B., 1998. The relationship between new production and vertical flux on the Ross Sea continental shelf. Journal of Marine Systems 17, 445–457.

Smith Jr., W.O., Gordon, L.I., 1997. Hyperproductivity of the Ross Sea (Antarctica) polynya during austral spring. Geophysical Research Letters 24, 233–236.

Smith Jr., W.O., Nelson, D.M., DiTullio, G.R., Leventer, A.R., 1996. Temporal and spatial patterns in the Ross Sea: phytoplankton biomass, elemental composition productivity and growth rates. Journal of Geophysical Research 101, 18455–18466.

Smith Jr., W.O., Nelson, D.M., Mathot, S. Phytoplankton growth rates in Ross Sea, Antarctica determined by independent methods: temporal variations. Journal of Plankton Research, in press.

Stoecker, D.K., Sieracki, M.E., Verity, P.G., Michaels, A.E., Haugen, E., Burkill, P.H., Edwards, E.S., 1994. Nanoplankton and protozoan microzooplankton during the JGOFS N. Atlantic Bloom Experiment. Journal of the Marine Biological Association of the United Kingdom 74, 427–443.

Stoecker, R.K., Gustafson, D.E., Verity, P.G., 1996. Micro- and mesoprotozooplankton at 140°W in the equatorial Pacific: heterotrophs and mixotrophs. Aquatic Microbial Ecology 10, 273–282.

Strom, S.L., Buskey, E.J., 1993. Feedings, growth, and behavior of the thecate heterotrophic dinoflagellate *Oblea rotunda*. Limnology and Oceanography 38, 965–977.

Strom, S.L., Welschmeyer, N.A., 1991. Pigment-specific rates of phytoplankton growth and microzooplankton grazing in the open subarctic Pacific Ocean. Limnology and Oceanography 36, 50–63.

Taylor, G.T., Haberstroh, P.R., 1988. Microzooplankton grazing and planktonic production in the Bransfield Strait observed during the RACER program. Antarctic Journal of U.S. 23, 126–128.

Tsuda, A., Kawaguchi, S., 1997. Microzooplankton grazing in the surface water of the Southern Ocean during an austral summer. Polar Biology 18, 240–245.

Verity, P.G., Stoecker, D.K., Sieracki, M.E., Nelson, J.R., 1993. Grazing growth and mortality of microzooplankton during the 1989 North Atlantic spring bloom at 47°N, 18°W. Deep-Sea Research 40, 1793–1814.

Verity, P.G., Stoecker, D.K., Sieracki, M.E., Nelson, J.R., 1996. Microzooplankton grazing of primary production at 140°W in the equatorial Pacific. Deep-Sea Research 43, 1227–1255.

Weisse, T., Scheffel-Möser, U., 1990. Growth and grazing loss rates in single-celled *Phaeocystis* sp. (Prymnesiophysceae). Marine Biology 106, 153–158.

Weisse, T., Tande, K., Verity, P., Hansen, F., Gieskes, W., 1994. The trophic significance of *Phaeocystis* blooms. Journal of Marine Systems 5, 67–79.