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Feeding ecology of the Antarctic krill, *Euphausia superba:* The role of phytoplankton community composition in the krill's diet

A dissertation submitted in partial satisfaction
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in

Ecology, Evolution and Marine Biology

by

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Haberman, K. L., R. M. Ross, and L.B. Quetin, 1993. "Palmer LTER: Grazing by the Antarctic krill *Euphausia superba* on *Nitzschia* sp. and *Phaeocystis* sp. monocultures." Antarctic Journal of the U.S. 28 (5): 217-219.

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FIELDS OF STUDY

Major Field: Ecology, Evolution and Marine Biology

Zooplankton ecophysiology
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Biological Oceanography
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Immunochemical Applications in Feeding Ecology
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ABSTRACT

Feeding ecology of the Antarctic krill, *Euphausia superba*: The role of phytoplankton community composition in the krill's diet

by

Karen Lynn Haberman

Phytoplankton blooms in the austral spring and summer are important food resources for the Antarctic krill. Major taxa comprising these blooms include diatoms, prymnesiophytes and cryptophytes. The relative contributions of these phytoplankton groups in the krill's diet were examined, with an emphasis on the prymnesiophyte *Phaeocystis*. Studies of grazing on *Phaeocystis* have produced conflicting results, and little is known about the trophic link between *Phaeocystis* and euphausiids.

Several approaches were included in this study. First, clearance and ingestion rates by *E. superba* on unialgal cultures of *Phaeocystis antarctica* and *Thalassiosira antarctica* were compared in the laboratory (Chapter 1). Egestion rates were also measured, and carbon and

nitrogen assimilation efficiencies were calculated (Chapter 2). Next, selectivity by krill for particular phytoplankton taxa was determined from grazing by krill on phytoplankton mixtures, using high performance liquid chromatography (HPLC) to track concentrations of taxon-specific photopigments (Chapter 3). Finally, the degree of grazing by krill on *Phaeocystis* was estimated with an immunochemical assay of stomach extracts from field-collected krill (Chapter 4).

Clearance and ingestion rates by krill on small *Phaeocystis antarctica* colonies (50-100 μm) were similar to those on *T. antarctica*, whereas rates on medium *P. antarctica* colonies (150-500 μm) and single cell *P. antarctica* were significantly lower than for *T. antarctica*. Mean carbon and nitrogen assimilation efficiencies for *P. antarctica* were 85% and 94%, respectively. Diatoms were selected over both prymnesiophytes (i.e. *Phaeocystis*) and cryptophytes in phytoplankton mixtures, even when *Phaeocystis* occurred as small colonies. Finally, krill grazed *Phaeocystis* at 20% of stations sampled, most of them nearshore.

My results suggest that Antarctic krill graze *Phaeocystis*, but to a lesser extent than they graze diatoms. This result should be considered when measuring and modeling food availability for Antarctic krill.

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CHAPTER 1

Grazing by the Antarctic krill, *Euphausia superba*, on *Phaeocystis antarctica* and *Thalassiosira antarctica*

ABSTRACT

Diatoms and prymnesiophytes are major constituents of spring phytoplankton blooms in the Antarctic. While diatoms are known to be well-grazed by krill, the role of prymnesiophytes in the krill's diet is unknown. Clearance and ingestion rates of *E. superba* grazing on three size classes of *Phaeocystis antarctica* were compared to rates on the diatom *Thalassiosira antarctica* in parallel, unialgal experiments. Clearance rates were calculated from disappearance of chlorophyll a during grazing, and converted to carbon and nitrogen ingestion rates based on elemental (CHN) analysis of phytoplankton cultures. Clearance and ingestion rates of krill on small *P. antarctica* colonies (50-100 μm) were similar to rates on *T. antarctica*, whereas rates on medium *P. antarctica* colonies (150-500 μm) and single cell *P. antarctica* were significantly lower than for *T. antarctica*. Overall, clearance rates on *P. antarctica* decreased with increasing colony size. Clearance rates on *T. antarctica* were variable, and correlated with degree of chain formation. These results suggest that *E. superba* effectively grazes small colonies of *P. antarctica*, but not larger colonies or single cells.

INTRODUCTION

The Antarctic krill, *Euphausia superba*, is the primary prey for a multitude of predators in Antarctic waters. In turn, phytoplankton is a primary food resource for the Antarctic krill. While krill may also consume zooplankton to an as yet unknown extent (Price et al., 1988; Atkinson and Snÿder, 1997; Pakhomov et al., 1997), growth and reproductive success of adult krill are presumably directly tied to the krill's ability to effectively graze spring phytoplankton blooms associated with the marginal ice zone (Ross and Quetin, 1986; Quetin and Ross, 1991). Diatoms are a major component of these blooms, and are also recognized as an important food source for krill because they are within the size range effectively grazed (Meyer and El-Sayed, 1983; Ishii et al., 1985; Quetin and Ross, 1985). However, prymnesiophytes, represented by the genus *Phaeocystis*, are also abundant in these blooms (Fryxell et al., 1988). In fact, recent studies show that *Phaeocystis* is often the dominant genus associated with spring ice edge blooms along the Antarctic Peninsula (Bidigare et al., 1996), where *E. superba* is abundant in shelf waters (Ross, et al., 1996). Furthermore,

Phaeocystis can also be a major component of near-shore, open water phytoplankton blooms in the Palmer Station region in spring and summer (Moline and Prézelin, 1996). Krill are frequently found in this coastal habitat (Lascara, et al. in press) which may be an important feeding ground for immature krill. On the basis of biomass and spatial distribution, *Phaeocystis* is a potentially important food resource for *E. superba*.

Two previous studies suggest that *Phaeocystis* is grazed by *Euphausia superba*. Sieburth (1960) found *Phaeocystis* in the guts of krill, providing qualitative evidence of grazing. More recently, Virtue et al. (1993) studied the ability of Antarctic krill to assimilate lipids from *Phaeocystis pouchetii*. Their data on lipid classes incorporated during the five month study show that krill can graze and assimilate *Phaeocystis*. However, no quantitative studies of grazing rate or assimilation efficiency by *E. superba* on *Phaeocystis* currently exist.

Hansen et al. (1994) conducted the only study to date that measured grazing rates of euphausiids on both *Phaeocystis* and diatoms. Clearance rates by *Thysanoessa* sp. on field-collected *Phaeocystis* were lower than its rate on cultured *Thalassiosira nordenskioldii*. The euphausiid tended to fragment *Phaeocystis* colonies into smaller

pieces, rather than ingesting them. However, mouthparts and feeding appendages of *Thysanoessa* sp. differ from *Euphausia* sp. (McClatchie and Boyd, 1983).

In contrast to the paucity of information on euphausiids, numerous studies have quantitatively examined the ingestion of *Phaeocystis* by other macro- or meso-zooplankton grazers, primarily copepods in northern waters (reviewed in Davidson and Marchant, 1992; Weisse, et al. 1994.) While these results were highly variable and method dependent, even within a species, some conclusions of possible relevance to *Euphausia superba* can be drawn. First, filter-feeding copepods may have greater difficulty grazing *Phaeocystis* colonies than do raptorially-feeding copepods (Miller and Hampton, 1989), and have been found with *Phaeocystis* fragments adhering to their appendages (Schnack, 1985). Krill may also have this problem, since they feed primarily by compression-filtration (Hamner, 1988) and their feeding basket is composed of fine setae (McClatchie and Boyd, 1983). Second, these studies demonstrated the importance of measuring grazing rates throughout the life cycle of *Phaeocystis*. Early in a *Phaeocystis* bloom, the alga occurs as flagellated, motile cells between 3-8 μm in diameter. As the bloom progresses, the proportion of the colonial form increases.

These colonies are composed of non-flagellated cells imbedded in a high carbohydrate "gelatinous" matrix (Verity et al., 1988). Progressively larger colonies (up to several millimeters) develop as the bloom continues. Ingestion of *Phaeocystis* by copepods appears to depend upon the phase of its life cycle and upon the size range and physiological state of the colonies (Estep et al., 1990; Weisse, et al., 1994). For example, Estep et al. (1990) showed that three species of *Calanus* were unable to graze actively growing *Phaeocystis* colonies, but consumed senescent ones. Since *E. superba* is known to graze more efficiently on larger phytoplankton (Meyer and El-Sayed, 1983; Quetin and Ross, 1985; Ishii et al., 1985), their ability to graze *Phaeocystis* may also depend upon its size and physiological state.

In summary, qualitative data from previous studies show that *Euphausia superba* grazes *Phaeocystis* to some degree, but grazing rates have not been quantified. Given the prominence of *Phaeocystis* in regions of high krill biomass, it is important to quantify the role of *Phaeocystis* as a food resource. Additionally, since the relative proportions of *Phaeocystis* and diatoms in these regions vary between seasons and years (Moline and Prézelin, 1996), studies of krill's ability

to graze *Phaeocystis* may help explain interannual variability in krill growth and reproduction (Quetin et al., 1996).

The objective of this study was to determine whether *Phaeocystis antarctica* in one or more of its forms is a significant food resource for *Euphausia superba*. To accomplish this goal, clearance and ingestion rates of *E. superba* on three size classes of *Phaeocystis antarctica* were compared to rates on a common and highly palatable diatom species, *Thalassiosira antarctica*.

METHODS

Collection and maintenance of krill. Experiments were conducted during the austral spring and summer of 1993-94, 1994-95, and 1996-97 at Palmer station, Antarctica, located on Anvers Island, west of the Antarctic Peninsula. Krill were collected either from a zodiac with a meter net, or from the M/V Polar Duke with a 2-m square-framed net. Mesh size was 1000-μm for nets and 500-μm for cod ends. All krill were maintained in 2.5-m diameter flow-through seawater tanks at Palmer Station. Ambient temperature in these tanks ranged from -0.5-1.5 °C. Feeding conditions depended upon the ambient phytoplankton

in the incoming seawater, and ranged from approximately 0.5-5.0 μg chl $a \cdot l^{-1}$. Krill were held in these tanks for a minimum of one week to ensure that krill used were healthy. Highest mortality rates occurred in the first few days after collection, presumably due to damage during trawling. Experimental animals were chosen from the holding tank on the basis of size (determined "by eye" to be 35-40 mm) and healthy appearance. A krill was considered healthy if it was actively swimming and/or feeding, displayed normal, wavelike pleopod movement during swimming, had a transparent abdomen without white patches, was not caught in the surface layer or lying on the bottom, and was not missing eyes. In 1996-97, an additional criterion was added. Only krill actively moving their feeding baskets were selected for experiments in order to minimize variability caused by individual differences in behavior.

Maintenance and assessment of phytoplankton cultures.

Phaeocystis antarctica and *Thalassiosira antarctica* cultures, both initially isolated from the Palmer Station region, were grown in 20-l clearboy containers at temperatures of 1.5-2.0 °C. Cool white fluorescent lights were used for illumination, with a light regime of 18

hours on, 6 hours off during the austral summer. Cultures were aerated using Silent Giant pumps, with air filtered through Gelman bacterial airvents to prevent contamination.

Thalassiosira antarctica was grown in f/2 media (Guillard and Ryther, 1962). *Phaeocystis antarctica* was grown primarily in f/2 media. However, specific media components were altered in the attempt to grow large, colonial *P. antarctica* in 1993-94 and 1994-95. Changes included: 1) alteration of media strength from f/2 to f/40 (Sieburth, 1960), 2) manipulation of relative manganese levels (Davidson and Marchant, 1987), and 3) manipulation of relative phosphate concentrations (Veldhuis and Admiraal, 1987). These variations in media strength and relative nutrient concentrations did not have any apparent effects on colony formation and size. In 1993-94, initial cultures of *P. antarctica* were composed entirely of single cells, which then developed into small, spherical or elliptical colonies approximately 50-100 μm which were used for experiments (Table 1). In 1994-95, all cultures of *P. antarctica* were composed entirely of single cells.

In an effort to produce larger *Phaeocystis antarctica* colonies during 1996-97, air flow into the clearboys was reduced in order to minimize

the degree of agitation inside the containers (Andrew Davidson, personal communication). The clearboys were not aerated for the initial 24 hours after culture inoculation, after which aeration levels were controlled by a bleed valve, so that air entered the clearboy at the rate of approximately 1 bubble/sec. This method produced medium-sized colonies with dimensions primarily between 100-500 μm (Table 1). Often the largest colonies had both a spherical and an elongate component and resembled volumetric flasks in shape. This low-level aeration technique also resulted in a sheet of *P. antarctica* on the bottom which would periodically dislodge and fragment. Sheet fragments produced this way were screened out with either a 500 μm or 1000 μm mesh prior to the experiment so that only true colonies were used in experiments.

Cultures were assessed bi-weekly. Cell and colony size and shape were evaluated microscopically. Cells were measured using either a Unitron inverted microscope or a Zeiss compound microscope. Preserved phytoplankton was sampled with a 1.0 ml Hensen-Stempel wide-bore syringe and transferred to a Sedgewick-Rafter counting chamber. For both phytoplankton species, 100 random particles (colonies of *Phaeocystis antarctica*, cells or chains of *Thalassiosira*

antarctica) were measured at their minimum and maximum dimensions. For *T. antarctica*, cell dimensions, and number and length of chains, were also quantified. Cultures were also checked for contamination by other phytoplankton species. In addition, duplicate samples of 10-50 ml were filtered onto GF/C filters, frozen, and analyzed for chlorophyll a (chl a) using a Turner Designs Model AU-005 digital fluorometer and the methods of Smith et al. (1981). During the 1996-97 season, additional samples of *P. antarctica* cultures that were mixtures of single cells and colonies were fractionated using a 20 μm filter which allowed single cells (3-8 μm diameter) to pass through. This allowed for measurement of chl a present in colonies only. These periodic chl a checks were used to determine growth rates of the phytoplankton.

Cultures were also sampled one day prior to each experiment for cell and colony measurements, chl a concentration and elemental analysis (Table 1). Subsamples of 100 ml from each culture were preserved in 0.35% glutaraldehyde/Lugol's solution (Rousseau et al., 1990) for microscopic analysis. Five replicate samples (10-50 ml depending upon estimated chl a concentration) were taken for chl a analysis as described above. Also, four subsamples, each of a different

volume, were filtered onto pre-combusted (500 °C, 1 hour) A/E filters. These filters were placed into pre-combusted (450 °C, 24 hours) aluminum sleeves and plates, stored in a 60 °C isotemp oven, and later analyzed for organic carbon (C) and nitrogen (N) on a Leeman Laboratories AE 440 CHN Analyzer. Linear regressions of C and N mass versus sample volume were calculated, and the slopes used to determine C and N concentrations of a culture. Values for chl a, C and N concentrations obtained in these analyses were used to calculate ratios of C:chl a, N:chl a and C:N (Table 1).

Experimental design. Grazing experiments were primarily conducted as paired comparisons between *Thalassiosira antarctica* and *Phaeocystis antarctica*. The purpose of this paired design was to minimize the confounding effects of krill feeding history, time of year, and subtle changes in experimental conditions which could contribute to differences in feeding rates unrelated to the phytoplankton type. While the major focus of this study was on the paired comparisons between *T. antarctica* and *P. antarctica*, results of additional, unpaired experiments with these species have been included in some analyses.

The range of phytoplankton concentrations used in these experiments was an important consideration. Both chl a and estimated

carbon concentrations were kept below the critical concentration as defined by Frost (1972, Fig. 1) above which the clearance rate decreases. These critical concentrations were estimated from previous studies of grazing by *Euphausia superba* (Price et al., 1988, Ross et al., 1998). Since clearance rates are at their maximum and relatively constant within this range, this choice allowed for paired comparisons of clearance rates even when chl a and/or carbon concentrations in the paired containers were similar but not equal.

A related question was which phytoplankton variable should be standardized for the different phytoplankton species. As the building block atom of organic molecules, carbon is roughly proportional to the total mass/energy of an organic substance, and would be the logical choice for this variable. In addition, carbon also appears to be a relevant parameter from the perspective of the functional response of a filter-feeder (Frost, 1972). However, it was not possible to measure carbon in the field, so chl a concentration was used as a proxy for phytoplankton concentration. During the 1993-94 and 1994-95 season, target chl a concentrations were equal for the paired containers. However, analyses from the first two years showed that C:chl a ratios for single cell and small, colonial *Phaeocystis antarctica* were

approximately double the C:chl a ratios for *Thalassiosira antarctica* (Tables 1 and 2). In an attempt to equalize carbon and keep *P. antarctica* concentrations within the range of maximum clearance rate, the initial chl a concentrations for *P. antarctica* treatments in 1996-97 were planned as half the values for *T. antarctica* treatments.

Experimental set-up and sampling. Experimental containers (47 cm diameter x 53 cm height) were filled with 40-45 l of filtered (0.45 μm) seawater and moved into the environmental room approximately 24 hours prior to the experiment to allow for temperature equilibration. The measured volume of phytoplankton required for each container, as calculated from the chl a measurements and the estimated growth rate of each culture, was added to the containers approximately 10 hours prior to the experiment. Additional filtered seawater was then added to the containers so that all containers held equivalent volumes (50-60 l depending upon the experiment). The phytoplankton was then mixed continuously with a plunger-type stirring system modified from Frost (1972). The containers were left in the stirring apparatus overnight to equilibrate to experimental temperature and light conditions (0.5 ±0.3 °C, dim light). Simultaneously, krill for the

experiments were removed from the maintenance tanks to the environmental room and kept overnight in two 20-l buckets of filtered seawater to acclimate to the physical environment prior to the experiment.

Each experiment lasted 12 hours, and was divided into two consecutive feeding periods of 6 hours (6-h) each. Identical, replicate containers were used for each feeding period, making four containers total for each paired experiment. The first feeding period was considered to be an acclimation period, since previous studies on filter-feeding copepods have found initial feeding rates to be abnormally high, presumably due to starvation and handling (Mullin, 1963). The first 6-h period was also used to monitor the phytoplankton concentration over time in the two containers without krill, which were subsequently used during the second feeding period as the experimental containers (Fig. 2).

During the first 6-h period, five 100-ml water samples were collected at 2-h intervals (hours 0, 2, 4 and 6) from the control containers (no krill), and at hours 0 and 6 from the containers with krill (Fig. 2). During the second six hour feeding period (hours 6-12), five 100 ml water samples were collected at 2-h intervals (hours 8, 10 and 12) from

the containers with krill. These samples were filtered and frozen for later chlorophyll analysis. In addition, a 100 ml sample was taken at hours 0, 6, and 12 from each container used for the control/second feeding period, and preserved in 0.35% glutaraldehyde/Lugols's for subsequent counts and measurements of cells and colonies. Krill were measured and weighed after the experiment.

For some experiments in 1996-97, there was only one pair of containers, and only a single feeding period. The first 6-h period was used as the control for phytoplankton growth with no krill grazing. During this time, krill remained in the 20-l buckets and were not acclimated to food levels as in the other experiments. Krill were transferred into the experimental containers at the beginning of the second 6-h period. Water samples were collected at 2-h intervals throughout the 12-h experiment.

Grazing rate calculations. Phytoplankton growth and grazing rates were calculated with best fit exponential regressions of time (t) and chlorophyll a concentration [chl a], based on the equations of Frost (1972). The [chl a] used was the average of the five replicates taken at each time period.

The phytoplankton growth coefficient (k) (Frost, 1972) was calculated from the control containers with no krill, where $[chl\ a]_0$ is the initial chlorophyll concentration and $[chl\ a]_t$ is the chlorophyll concentration at time t (in hours).

$$(1)\ k = \ln([chl\ a]_t/[chl\ a]_0)*t^{-1}$$

The experimental phytoplankton loss coefficient, b, defined as rate of change in phytoplankton due to both growth and grazing, was calculated from the containers with krill, both with and without acclimation to the food.

$$(2)\ b = \ln([chl\ a]_t/[chl\ a]_0)*t^{-1}$$

The grazing coefficient (g) (Frost, 1972) was calculated from the phytoplankton growth coefficient and phytoplankton loss coefficients:

$$(3)\ g = k-b$$

Grazing coefficients were then converted to clearance rates (F in l•g wet $wt^{-1}•h^{-1}$), i.e. the wet-weight specific rate at which a given volume of seawater is swept clear of phytoplankton by krill (Frost, 1972).

$$(4)\ F=g•V/W.$$

V is the volume of the phytoplankton mixture in the experimental containers and W is the wet weight of krill per container.

Carbon ingestion rates (I_C) and nitrogen ingestion rates (I_N) were calculated from these clearance rates with the equations of Marin et al. (1986) and the C:chl a ratios and N:chl a ratios determined for the cultures:

$$(6)\ I_C = F^*[\text{chl a}]_0^*(\text{C:chl a})$$

$$(7)\ I_N = F^*[\text{chl a}]_0^*(\text{N:chl a})$$

The above calculations assumed: 1) F did not change during each 6 h feeding period, 2) entire cells and colonies were ingested, and 3) C:chl a ratios and N:chl a ratios remained constant during the experiment.

The first assumption was tested with data from 1996-97 experiments by calculating F for each 2-h interval during the first feeding period with equations 1-4, and testing for differences in clearance rates among intervals.

These calculated ingestion rates were considered as initial rates of grazing and used to compare grazing on different phytoplankton types, not to calculate actual grazing loss over the course of the experiment, which was measured directly. Such a calculation would overestimate ingestion rates for containers which had substantial (>14%) decreases in phytoplankton over the 6-h feeding period (Marin, et al., 1986).

RESULTS

Comparison of clearance rates for the first and second feeding periods.

Clearance rates during the first and second feeding periods of each experiment were compared with a Wilcoxon signed rank test. The purpose of this test was to determine whether the two periods could be treated as duplicate experiments, even though krill had been acclimated to food conditions prior to the second, but not the first,

feeding period. When experiments for all years were included, clearance rates by *Euphausia superba* on *Thalassiosira antarctica* were significantly higher for the first feeding period than the second feeding period (Wilcoxon signed rank test, p=0.035). When individual years were considered, two of the three years followed this pattern. However, in 1993-94 the pattern was reversed: *E. superba* had lower clearance rates on *T. antarctica* for the first feeding period in 75% of the experiments. Clearance rates on *Phaeocystis antarctica* were significantly higher for the first feeding period in all years combined (WSRT, p=0.002), and this result was consistent among years. These analyses showed that the two feeding periods were not equivalent. The first feeding period was used in all further analyses presented here, since some experiments included only the first feeding period. However, results for the second feeding period were generally qualitatively consistent with these results (Haberman, unpublished data).

Given the differences between clearance rates for the first and second feeding periods, it was important to test whether clearance rates were consistent during the entire 6-h period. Accordingly, clearance rates for the three 2-h intervals within the first feeding period were

compared. These data were available only for the 1996-97 experiments (n=7). Clearance rates among the three 2-h intervals were not significantly different from each other for either *Thalassiosira antarctica* (Friedman 2-way ANOVA, p=1.0) or *Phaeocystis antarctica* (p=0.15). Therefore, the 6-h period was appropriate to use for comparing clearance rates in these experiments.

Pairwise comparisons of clearance rates between *Thalassiosira antarctica* and *Phaeocystis antarctica*.

Experiments were analyzed as paired comparisons of clearance rates by *Euphausia superba* on *Thalassiosira antarctica* and *Phaeocystis antarctica*. Since the size of *P. antarctica* was relatively consistent within a year, experimental pairs were grouped by year to compare clearance rates for each size category of *P. antarctica* (small colonies, medium colonies, and single cells) with *T. antarctica*. A Wilcoxon signed rank test was used to test for differences in clearance rates between *T. antarctica* and *P. antarctica*, and $p<0.05$ was considered significant.

Single cell *Phaeocystis antarctica*. Clearance rates by krill on single cell *Phaeocystis antarctica* were significantly lower than on *Thalassiosira antarctica* (p=0.03, Fig. 3a, Table 3). The mean clearance rate for *P. antarctica* was nearly an order of magnitude less than the mean rate for *T. antarctica.*

Colonial *Phaeocystis antarctica.* Clearance rates by *Euphausia superba* on small *Phaeocystis antarctica* colonies were lower than on *Thalassiosira antarctica* in four out of five pairings (Fig. 3b). However, these differences were not significant (p=0.23, Table 3). The mean clearance rate for *P. antarctica* was approximately 70% of the mean for *T. antarctica* (Table 3).

Clearance rates by krill on medium *Phaeocystis antarctica* colonies were significantly less than on *Thalassiosira antarctica* (Fig. 3c and Table 3, p=0.02). The mean clearance rate for *P. antarctica* was less than 30% of the mean rate for *T. antarctica.*

Spearman rank correlation coefficient. In addition to testing for differences between *Thalassiosira antarctica* and *Phaeocystis antarctica*

clearance rates in paired experiments, correlations between *T. antarctica* and *P. antarctica* clearance rates were also calculated using the Spearman rank correlation (Siegel, 1956). This correlation is indicative of the effects of individual experimental conditions on clearance rates, independent of phytoplankton type. This correlation was found to be highly significant for the 1996-97 paired experiments ($p<0.01$,Table 3). However, there were no significant correlations for the 1993-94 or 1994-95 seasons.

Interannual comparisons of clearance rates

Clearance rates were first analyzed as a function of initial carbon concentrations to test the assumption that initial phytoplankton concentrations were below the critical concentration. These tests were necessary to determine the appropriate method for comparing experiments with different initial concentrations.

Clearance rates on *Thalassiosira antarctica* were not a function of initial carbon concentration for any of the three years (regression analysis, $p>0.20$ all years). Clearance rates on *Phaeocystis antarctica* were also not a function of initial carbon concentration (regression analysis,

$p>0.20$ all years). These results suggest that the attempt to make initial chl a concentrations below the critical concentration for all experiments was successful. Clearance rates among years for *T. antarctica*, and among different size categories of *P. antarctica*, were compared with Tukey HSD multiple comparison tests.

Thalassiosira antarctica. Clearance rates by *Euphausia superba* on *Thalassiosira antarctica* were compared between years in order to assess interannual variability in clearance rates independent of phytoplankton type. Clearance rates during the 1993-94 season and the 1996-97 season were similar. However, clearance rates differed significantly between the 1993-94 and 1994-95 seasons (Tukey HSD multiple comparisons, Table 4). The mean clearance rate for *T. antarctica* in 1993-94 was nearly double the mean clearance rate in 1994-95 (Table 4).

Thalassiosira antarctica cultures were grown under similar conditions within and between years; however, the precise characteristics of the *T. antarctica* cultures were variable. The clearest interannual differences were in cell size and chain formation (Table 1). During 1993-94, cells were primarily small (20-30 μm) and occurred in

mixtures of single cells and two-cell chains. During 1994-95, cells were either mostly large (50-60 μm) and single or mostly small and single, depending upon the specific culture and experiment. During 1996-97, cells were primarily small and single. There was no significant correlation between clearance rates and cell size for either of the two feeding periods during 1994-95, the only year when significant numbers of large cells were grown. In contrast, degree of chain formation was significantly correlated with clearance rates for the first feeding periods during both 1993-94 and 1996-97, and weakly correlated with clearance rates during 1994-95 (Fig. 4). These results suggest that degree of chain formation may affect clearance rates.

Although cultures in exponential growth phase were used for experiments, specific phytoplankton growth rates varied among cultures. However, no correlation was found between specific growth rates and clearance rates (regression, r^2=0.03, p=0.59) in 1994-95, the year with highest variability for both these rates. Finally, there were no significant differences among years for either C:N or C:chl a (Table 2) so these factors were apparently not linked with interannual variability in *T. antarctica* clearance rates.

Phaeocystis antarctica. Clearance rates by *Euphausia superba* on small *Phaeocystis antarctica* colonies were significantly greater than clearance rates on both medium *P. antarctica* colonies and single cell *P. antarctica* (Tukey HSD multiple comparisons, Table 5). The mean clearance rate for small colonies was three times the rate for medium colonies, and ten times the rate for single cells. The outlier for 1996-97 (Expt 6-02) was not included in this analysis because its size was intermediate between the small and medium colony size categories (Table 1).

Colony size varied among experiments within each category. To more closely examine the factor of colony size, clearance rate was plotted as a function of the median value of the maximum colony dimension (Table 1) for all experiments with colonial *Phaeocystis antarctica* (Fig. 5). Clearance rate significantly decreased with increasing colony size ($y = -0.004x + 1.11$, $r^2=0.71$, $P<0.001$).

Since *Phaeocystis* colony size is sometimes correlated with C:N and/or C:chl a (Davies et al., 1992), additional analyses were conducted to test whether the significant relationship between colony size and clearance rate was confounded by these factors. Regression analysis

found no significant correlation between colony size and either C:N ($p=0.63$) or C:Chl a ($p=0.70$).

Interannual comparison of carbon and nitrogen ingestion rates

Thalassiosira antarctica. Carbon and nitrogen ingestion rates by *Euphausia superba* on *Thalassiosira antarctica* during the first feeding period were plotted as functions of initial carbon concentration in each of the three experiment years (Fig. 6). Both carbon and nitrogen ingestion between years showed considerable overlap; the slopes were homogenous and there were no significant differences in the regression between years for either carbon ingestion rates (ANCOVA, $p=0.19$) or nitrogen ingestion rates ($p=0.42$). Overall, there was a highly significant correlation between carbon ingestion rates and initial carbon concentration ($y = 1.1 x - 42$, $r^2 = 0.67$, $P<0.001$, Fig. 6a), as well as between nitrogen ingestion rates and initial carbon concentration ($y = 0.29 x - 16$, $r^2 = 0.63$, $P<0.001$, Fig. 6b).

Phaeocystis antarctica. Carbon and nitrogen ingestion rates for each size category of *Phaeocystis antarctica* were analyzed a functions of

initial carbon concentration. In contrast to *T. antarctica,* neither carbon nor nitrogen ingestion rates for *P. antarctica* were significantly correlated with initial carbon concentration for any size category of *P. antarctica*. Both carbon and nitrogen ingestion rates for small *P. antarctica* colonies were significantly higher than for either medium *P. antarctica* colonies or single cell *P. antarctica* (Tukey HSD multiple comparisons, Table 6). While carbon and nitrogen ingestion rates for all size categories of *P. antarctica* overlap with *T. antarctica* rates at low initial carbon concentrations (Fig. 7), lack of significant correlation of ingestion rate and initial carbon concentration for *P. antarctica* leads to an increasing divergence of the rates for the two species as carbon concentrations increase, especially for medium colonies and single cells.

DISCUSSION

Pairwise comparison of clearance rates.

Pairwise comparisons showed that clearance rates on *Phaeocystis antarctica* were generally lower than on *Thalassiosira antarctica,* and dependent upon size. Pairwise comparisons directly compared the two phytoplankton species, and minimized the effects of variables such as behavior, health of the krill and seasonal changes, which could not be controlled during the experiments. The actual utility of this paired design was tested with the Spearman rank correlation coefficient. The highly significant Spearman correlation for 1996-97 implied that some aspect of the experimental conditions common to both containers was variable and strongly affected the clearance rate. During 1996-97, the temperature-controlled room experienced a greater degree of human activity than in previous years. This increased activity, coupled with brief, accidental changes in light levels may have influenced the krills' behavior.

One important aspect of the paired design was the requirement to match the two phytoplankton species in a relevant way. This was difficult to accomplish for several reasons. 1) The most relevant variable is not clear (Rapport, 1981). 2) The most conservative variable to match, carbon, could not be measured in the field. 3) The easiest variable to measure, chl a, is not necessarily correlated with available energy or some other aspect of food value. While Cowles (1988) used chl a to determine food value within a species, based on the correlation between chl a per cell and growth rate, there is no clear reason for it to reflect food value between species. 4) The two phytoplankton types had significantly different C:chl a ratios. Chl a was reasonably well-matched for the pairings of *T. antarctica* and both the small *P. antarctica* colonies and single cell *P. antarctica* (Table 1), but differences in their C:chl a ratios created a mismatch for carbon. Attempts were made to take C:chl a ratios into account and match carbon more closely for pairings between *T. antarctica* and medium *P. antarctica* colonies. These attempts were not always successful, due to the high variability of C:chl a ratios.

The use of clearance rate, rather than ingestion rate, for these paired experiments circumvented the need to precisely match the

phytoplankton concentrations. Since clearance rates were not correlated with initial carbon concentration over the range of values in the paired containers, the results for these paired experiments are valid even in cases where there were significant concentration differences for relevant variables between paired tubs. Also, evaluation of the 2-h intervals for 1996-97 showed the consistency of clearance rates throughout the 6-h feeding period, even as phytoplankton concentration dropped over time.

Interannual comparisons of clearance and ingestion rates.

Interannual comparisons of krill's clearance, carbon ingestion and nitrogen ingestion rates on *Phaeocystis antarctica* showed that krill grazed small colonies at significantly greater rates than either medium colonies or single cells. However, *Thalassiosira antarctica* was considered the standard phytoplankton species against which *P. antarctica* was compared; therefore, the *P. antarctica* results should be examined in light of the *T. antarctica* results. Accordingly, the first part of this section is devoted to interannual comparisons of grazing on *T.*

antarctica, after which the different size categories of *P. antarctica* are discussed.

Thalassiosira antarctica. Significant interannual differences were found for clearance rates on *Thalassiosira antarctica*. Possibly, these differences were a result of differences in chain formation between years. The strongest correlations between chain formation and clearance rates were found during 1993-94 and 1996-97, the two seasons when *T. antarctica* was comprised of small cells (20-30 μm diameter) almost exclusively. For those years, a higher percentage of cells in chains meant more relatively large particles (maximum dimension 50-60 μm for two cell chains), consistent with the relationships between size and clearance found in other studies (Quetin and Ross, 1985). On the other hand, cultures of predominantly 2-cell chains in 1993-94 were grazed at higher rates than large, single *T. antarctica* cells grown in 1994-95, even though the linear dimensions of the 2-cell chains and the large, solitary cells were similar. Furthermore, there was no difference between clearance rates for single *T. antarctica* cells of different sizes but the same overall shape. Perhaps, chains were more effectively retained within the feeding basket, due to their geometry. In two previous

studies, *Euphausia* spp. also exhibited higher clearance rates on chain-forming diatoms than on solitary cells (Stuart, 1989; Meyer and El-Sayed, 1993).

Food quality, measured as relative concentrations of carbon, nitrogen, and chl a, did not appear to be a factor. C:N and C:chl a ratios for *Thalassiosira antarctica* were quite similar among years (Table 2). Possibly, a more subtle aspect of food quality, such as lipid or protein composition, varied between years.

Factors other than phytoplankton variability may have played a role. For example, some aspect of krill health or feeding history may have reduced grazing rates during 1994-95.

Phaeocystis antarctica. Single cell *Phaeocystis antarctica* (1994-95) was both cleared and ingested at significantly lower rates than small *P. antarctica* colonies (1993-94) (Tables 5 and 6). However, the significant difference between *Thalassiosira antarctica* clearance rates for the same years suggests that the *P. antarctica* differences could be due to lower grazing rates overall in 1994-95, rather than a real difference between the two *P. antarctica* size categories. The paired analyses can be used to clarify this result. During 1994-95, single *P. antarctica* was cleared at a

rate nearly an order of magnitude below the *T. antarctica* for the first feeding period, whereas during 1993-94, clearance rates on small *P. antarctica* colonies could not be distinguished from *T. antarctica* (Table 3). Therefore, the clearance rate differences between single cell and small, colonial *P. antarctica* are likely due to differences in the *P. antarctica*, and not to interannual differences in clearance rates. These results are consistent with previous studies on phytoplankton size and ingestion. Cells in the size class of single cell *Phaeocystis* (<10 μm) are less effectively grazed, probably due to reduced sieving efficiency of the feeding basket for these small particles (McClatchie and Boyd, 1983; Quetin and Ross, 1985; Ishii, 1985).

Medium *Phaeocystis antarctica* colonies (1996-97) were also cleared and ingested at lower rates than small *P. antarctica* colonies (Tables 5 and 6). Since clearance rates on *Thalassiosira antarctica* for 1993-94 and 1996-97 were similar, these results were presumably due to some physical or physiological difference between small and medium colonies.

Why did the krill clear and ingest the medium *Phaeocystis antarctica* colonies at lower rates than the small ones? Based on the regression of clearance rate and colony size (Fig. 5), the relevant factor

may be size itself. However, krill are certainly capable of ingesting much larger particles. For example, Price et al. (1988) showed successful grazing by krill on copepods, a larger food item than the colonial *P. antarctica* used in this study. When preying on copepods, the krill appeared to feed raptorially rather than by filtering. Possibly, the medium colonies in this experiment are between the size that krill can efficiently capture through either raptorial feeding or filtration.

Food quality is another important factor which influences grazing rates. For example, Cowles (1988) demonstrated that the copepod *Acartia tonsa* grazed fast-growing cells with relatively low C:N and C:chl a ratios at higher rates than slow growing cells of the same species with higher C:N and C:chl a ratios. In this study, neither C:chl a nor C:N ratios were correlated with *Phaeocystis antarctica* colony size. Verity et al. (1988) also found that extracellular:cellular ratios of carbon were not strictly tied to colony size for nutrient replete colonies. Van Rijssel et al. (1997) showed that *Phaeocystis globosa* was a hollow ball with a thin layer of cells and mucopolysaccharide matrix, rather than a solid sphere filled with mucopolysaccharides as other studies had indicated (Rousseau, 1990). This structural characteristic explains why C:chl a and C:N were not strictly tied to colony size. On the other hand,

the relatively large coefficient of variation of C:N ratios for medium colonies suggests that their quality was more variable than small colonies.

Optimally foraging animals spend more time searching than feeding if food quality is poor (MacArthur and Pianka, 1966). In the field, C:N ratios of *Phaeocystis* blooms often increase when large colonies predominate (Davies et al., 1992). If the nutritional value of larger *Phaeocystis* colonies is more variable than small colonies and clearly lower than diatoms, it may be advantageous for the krill to limit their intake of larger *Phaeocystis* and instead search for more consistently nutritional food resources.

It is also possible that the medium *Phaeocystis antarctica* colonies clogged the krill's feeding basket, as has been found for other filter feeding invertebrates (Pieters et al., 1980; Schnack, 1985). Microscopic examination of the krill's feeding basket for *P. antarctica* particles adhering to the setae would help answer this question. This could be coupled with TEP techniques (Alldredge et al., 1993) to detect transparent polysaccharide particles which might also interfere with feeding.

The role of *Phaeocystis* spp. in the krill's diet

In these experiments, small colonial *Phaeocystis antarctica* was cleared and ingested at rates similar to *Thalassiosira antarctica*. Thus, it appears possible for krill to benefit from *Phaeocystis* blooms when *Phaeocystis* exists primarily as small colonies. However, these results do not necessarily mean that *Phaeocystis* colonies are nutritionally equivalent to diatoms.

In particular, the nature of the carbon which comprises *Phaeocystis* colonies is different than diatoms. The C:chl a ratios of the small *Phaeocystis antarctica* colonies were approximately 40% higher than for single cell *P. antarctica*, with the extra carbon presumably extracellular, and composed of mucopolysaccharides which are probably low in nutritional value compared to diatom carbon (Sargent, 1985). Also, since C:N ratios are similar for *P. antarctica* single cells and colonies, a significant proportion of the nitrogen in colonies is probably associated with the extracellular matrix. The form of this nitrogen is not known (Verity et. al, 1988) nor is its ability to be assimilated by the krill. Furthermore, overall C:N ratios were greater in all three size categories

of *P. antarctica* than in *Thalassiosira antarctica* (Table 2). This may be indicative of low food quality relative to diatoms. Assimilation and growth studies would help elucidate the ability for *Phaeocystis* carbon and nitrogen to be effectively utilized by krill.

Another factor to consider is the low lipid level of *Phaeocystis.* In particular, *P. pouchetii* is low in essential fatty acids (Nichols et al., 1991). Virtue (1993) fed Antarctic krill diets of *P. pouchetii* or *Phaeodactylum tricornutum* and found the krill's lipid percentage and composition to be equivalent for the two food sources. However, *Phaeodactylum* sp. is known to be a poor food source for crustacean grazers in comparison to diatoms (Brown et. al, 1989 as cited in Virtue et al., 1993), and lipid values for the fed krill in the Virtue study were similar to winter field-collected animals which presumably had not fed (Ross and Quetin, unpublished data).

Finally, this study did not address selectivity by krill on mixtures of *Phaeocystis* and diatoms, even though they commonly co-occur at ice-edge blooms (Fryxell et al., 1988). Such studies are necessary to assess the relative utilization of prymnesiophytes and diatoms by krill in Antarctic waters.

CONCLUSION

Consistent and significant differences between grazing rates by krill on *Thalassiosira antarctica* and *Phaeocystis antarctica,* as well as among the different size classes of *P. antarctica,* were found in this study. These differences were seen alongside high variability in grazing rates on *T. antarctica.* Grazing on *P. antarctica* was dependent upon size: while small *P. antarctica* colonies were cleared and ingested at rates roughly equivalent to *T. antarctica,* medium colonies and single cells of *P. antarctica* were cleared and ingested at relatively low rates. These results suggest that krill can take advantage of *P. antarctica* blooms in the field, when they are primarily comprised of small colonies. However, as colony size increases, or if single cells comprise a large proportion of the bloom, the role of *Phaeocystis* as a food source for krill would be markedly reduced.

Mechanisms underlying size selectivity for *Phaeocystis antarctica* colonies should be more fully examined. Differences in handling time, feeding basket blockage, nutritional quality of phytoplankton, and behavior of krill may all result in low clearance and ingestion rates,

and distinguishing between these differences would lead to a further understanding of the krill's ability to exploit different food resources in the field. Additionally, studies of assimilation and nutrition, as well as selectivity by the krill in mixed blooms, are necessary to clarify the role of *Phaeocystis* as a food source for Antarctic krill.

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Table 1. Initial experimental conditions. T.a.: *Thalassiosira antarctica*, P.a.: *Phaeocystis antarctica*. Phytoplankton size and elemental ratios (C:N, C:chl a) were obtained from culture assessments one day prior to the experiment. T. a. cell size refers to the diameter range of the dominant cell type. S: 20-30 µm, L: 50-60 µm. % chains: percent of T.a. particles which are chains, not individual cells. Initial chlorophyll concentrations (µg/l) were measured at the beginning of the experiment, and initial carbon concentrations (µg/l) were calculated from intial chlorophyl concentration and C:chl a. Numbers in bold indicate values for paired experiments, while others are additional, unpaired experiments. Expt. 6-01 was run as a paired experiment but not evaluated as one; due to the extremely high carbon values for P. a., P.a. was eliminated from all analyses, but T.a. was used in "additional" analyses as described in the text. An * indicates experiments where one T. a. container was paired with two P. a. containers. The T.a is listed twice in those cases.

YEAR	EXPT	MIN DIMENSION (median value)		MAX DIMENSION (median value)		ADDITIONAL INFO T. a.		C: N		C: chl a		Chl a Initial, µg/l		Carbon Initial, µg/l	
		T. a.	P. a.	T.a.	P.a.	cell size	% chains	T. a.	P. a.	T. a.	P. a.	T. a.	P. a.	T. a.	P. a.
1993-94	**3-05**	**25**	**58**	**30**	**67**	**S**	**50**	**10.1**	**6.6**	**46**	**125**	**2.9**	**2.8**	**134**	**344**
1993-94	**3-08**	**24**	**86**	**45**	**106**	**S**	**56**	**4.0**	**4.5**	**25**	**45**	**4.6**	**3.7**	**114**	**165**
1993-94	**3-09**	**17**	**48**	**25**	**58**	**S**	**32**	**4.4**	**5.4**	**26**	**55**	**3.5**	**3.9**	**92**	**216**
1993-94	**3-11**	**25**	**48**	**46**	**58**	**S**	**71**	**4.6**	**6.2**	**24**	**94**	**5.0**	**4.1**	**121**	**383**
1993-94	**3-12**	**25**	**67**	**43**	**77**	**S**	**83**	**4.8**	**6.7**	**24**	**80**	**6.5**	**5.7**	**155**	**456**
1993-94	3-10	24		47		S	66	4.4		25		4.7		119	
1993-94	3-14(1)	25		44		S	53	5.2		29		8.3		239	
1993-94	3-14(2)	25		47		S	55	4.7		30		5.8		174	
1994-95	**4-02**	**15**	**7**	**20**	**7**	**S**	**0**	**4.3**	**5.0**	**40**	**43**	**2.6**	**2.9**	**101**	**124**
1994-95	**4-04**	**23**	**7**	**52**	**7**	**L**	**5**	**4.3**	**4.7**	**31**	**66**	**4.1**	**4.7**	**128**	**313**
1994-95	**4-09**	**16**	**7**	**21**	**7**	**S**	**5**	**4.7**	**5.1**	**21**	**50**	**5.6**	**5.1**	**119**	**254**
1994-95	**4-10**	**18**	**7**	**20**	**7**	**S**	**8**	**4.7**	**5.1**	**24**	**50**	**6.8**	**5.1**	**162**	**251**
1994-95	**4-11**	**17**	**7**	**20**	**7**	**S**	**4**	**5.0**	**5.4**	**25**	**43**	**6.2**	**5.8**	**154**	**248**
1994-95	**4-15**	**16**	**7**	**21**	**7**	**S**	**17**	**4.8**	**7.4**	**27**	**63**	**6.9**	**5.8**	**185**	**364**
1994-95	4-01		7		7				5.1		43		2.8		121
1994-95	4-05(1)	23		50		L	11	4.6		49		6.8		333	
1994-95	4-05(2)	23		51		L	5	4.9		22		4.9		110	
1994-95	4-07(1)	20		52		L	8	4.4		25		6.2		153	
1994-95	4-07(2)	21		52		L	6	4.3		25		5.4		131	
1994-95	4-12	15		19		S	5	6.3		38		5.6		209	
1994-95	4-14(1)	16		19		S	2	5.3		30		6.9		206	
1994-95	4-14(2)	20		48		L	6	4.8		23		5.4		122	

Table 1 (continued)

YEAR	EXPT	MIN DIMENSION (median value)		MAX DIMENSION (median value)		ADDITIONAL INFO T. a.		C: N		C: chl a		Chl a Initial, µg/l		Carbon Initial, µg/l	
1996-97	6-02	17	106	31	125	S	26	3.9	5.2	26	78	17.4	11.4	458	884
1996-97	6-05	19	96	29	173	S	2	4.4	14.0	28	124	17.9	4.5	501	552
1996-97	6-06 A	18	144	29	192	S	5	4.2	15.2	27	126	8.2	3.3	226	415
1996-97	6-06 B*	18	154	29	259	S	5	4.2	6.0	27	65	8.2	3.0	226	196
1996-97	6-07	18	144	29	192	S	5	4.2	6.0	27	65	10.8	2.6	297	172
1996-97	6-08 A	19	173	28	298	S	6	4.3	5.8	24	67	9.5	7.8	231	517
1996-97	6-08 B*	19	173	28	298	S	6	4.3	5.8	24	67	9.5	3.6	231	241
1996-97	6-01	17	106	31	106	S	12	3.7	63	26	173	20.1	25.7	525	4435
1996-97	6-04	25		63		S	16	5.1		49		10.6		518	

Table 2. a) C:N statistics and b) C:Chl a statistics for all phytoplankton, including means, standard deviation (SD), coefficient of variation (SD/mean) and Tukey HSD Multiple Comparison probabilities. P-values are listed in matrix; $p<0.05$ was considered signficant and indicated with an asterisk (*). T.a.= *Thalassiosira antarctica*. P.a.=*Phaeocystis antarctica*. sm col=small colonies, m col=medium colonies, single=single cells.

a)	MEAN	±SD	C.V.	T.a. 93-94	T.a. 94-95	T.a. 96-97	P.a. single	P.a. sm col	P.a. m col
T.a. 93-94	4.60	±0.37	0.08	1.000					
T.a. 94-95	4.81	±0.54	0.11	1.000	1.000				
T.a. 96-97	4.26	±0.45	0.11	0.999	0.985	1.000			
P.a., single	5.38	±0.89	0.16	0.960	0.982	0.838	1.000		
P.a., sm. col	5.86	±0.94	0.16	0.824	0.866	0.635	0.997	1.000	
P.a., m col	8.34	±4.29	0.51	0.004*	0.002*	0.001*	0.034*	0.177	1.000

b)	MEAN	±SD	C.V.	T.a. 93-94	T.a. 94-95	T.a. 96-97	P.a. single	P.a. sm col	P.a. m col
T.a. 93-94	28.7	±7.3	0.25	1.000					
T.a. 94-95	29.1	±8.3	0.29	1.000	1.000				
T.a. 96-97	29.8	±8.4	0.28	1.000	1.000	1.000			
P.a., single	50.9	±10.0	0.16	0.002	0.220	0.385	1.000		
P.a., sm. col	79.7	±31.9	0.40	0.001*	0.000*	0.002*	0.172	1.000	
P.a., m col	99.7	±42.0	0.42	0.000*	0.000*	0.000*	0.001*	0.548	1.000

Table 3. Clearance rate ($l \cdot g$ wet $wt^{-1} \cdot h^{-1}$) values and statistics for paired experiments. P.a.: *Phaeocystis antarctica*. T.a.: *Thalassiosira antarctica*. P.a. size categories: SM COL:small colonies, MCOL:medium colonies, SINGLE: single cells. See text and Table 1 for further size information.WSRT: P-values for Wilcoxon's signed ranked test; an * indicates a signficant difference ($p<0.05$) in clearance rates. All signficant values indicate a lower clearance rate for P.a.. Spearman's corr. coef: Spearman rank correlation coefficient, rho. The ** indicates $p<0.01$, based on Table P in Siegel, 1956. Test statistics are not given for M COL, feeding period 2, because there were too few pairs.

Experiment information				**Clearance rates**				**Test statistics**	
P.a. size category	Year	Total pairs	Feeding period	T.a. Mean	T.a. SE	P.a. Mean	P.a. SE	WSRT p-value	Spearman corr. coef.
SINGLE	1994-95	6	1	0.68	0.18	0.09	0.04	0.03*	0.12
SM COL	1993-94	5	1	1.27	0.28	0.91	0.12	0.23	-0.67
M COL	1996-97	7	1	1.10	0.44	0.30	0.07	0.02*	0.93**

Table 4. Clearance rates ($l \bullet g$ wet $wt^{-1} \bullet h^{-1}$) for *Euphausia superba* on *Thalassiosira antarctica* (T.a.) Included are mean, standard deviation (SD) and Tukey HSD Multiple Comparison probabilities listed as p-values; $p<0.05$ was considered signficant and indicated with an asterisk (*).

Year	MEAN	±SD	T.a. 93-94	T.a. 94-95	T.a. 96-97
93-94	1.23	±0.50	1.000		
94-95	0.69	±0.37	0.035*	1.000	
96-97	0.92	±0.54	0.440	0.547	1.000

Table 5. Clearance rates ($l \cdot g$ wet $wt^{-1} \cdot h^{-1}$) for *Euphausia superba* on *Phaeocystis antarctica* (P.a.) small colonies (sm col), medium colonies (m col) and single cells (single). Included are mean, standard deviation (SD) and Tukey HSD Multiple Comparison probabilities listed as p-values; $p<0.05$ was considered signficant and indicated with an asterisk (*).

Phytoplankton Type	MEAN	±SD	P.a. single	P.a. sm col	P.a. m col
P.a. single	0.08	±0.09	1.000		
P.a. sm col	0.90	±0.26	0.000*	1.000	
P.a. m col	0.30	±0.19	0.095	0.000*	1.000

Table 6. a) Carbon ingestion rates (µg C•g wet wt^{-1}•h^{-1}) and b) Nitrogen ingestion rates (µg N•g wet wt^{-1}•h^{-1}) for *Euphausia superba* on *Phaeocystis antarctica* (P.a.) small colonies (sm col), medium colonies (m col) and single cells (single). during the first feeding period. Included are mean, standard deviation (SD) and Tukey HSD Multiple Comparison probabilities listed as p-values; $p<0.05$ was considered signficant and indicated with an asterisk (*).

a)

Phytoplankton type	MEAN	±SD	P.a. single	P.a. sm col	P.a. m col
P.a. single	21	±17	1.000		
P.a. sm col	266	±71	0.000*	1.000	
P.a. m col	128	±121	0.070	0.030*	1.000

b)

Phytoplankton type	MEAN	±SD	P.a. single	P.a. sm col	P.a. m col
P.a. single	4.3	±3.7	1.000		
P.a. sm col	45.0	±8.5	0.001*	1.000	
P.a. m col	18.9	±24.4	0.220	0.030*	1.000

Fig. 1. **Modified figure 6 from Frost (1972) depicting the theoretical functional response curve for filter feeding copepods.** The dark bar on x-axis is the carbon concentrations for which clearance rates are maximum. The arrow points to the critical carbon concentration, above which clearance rates decline.

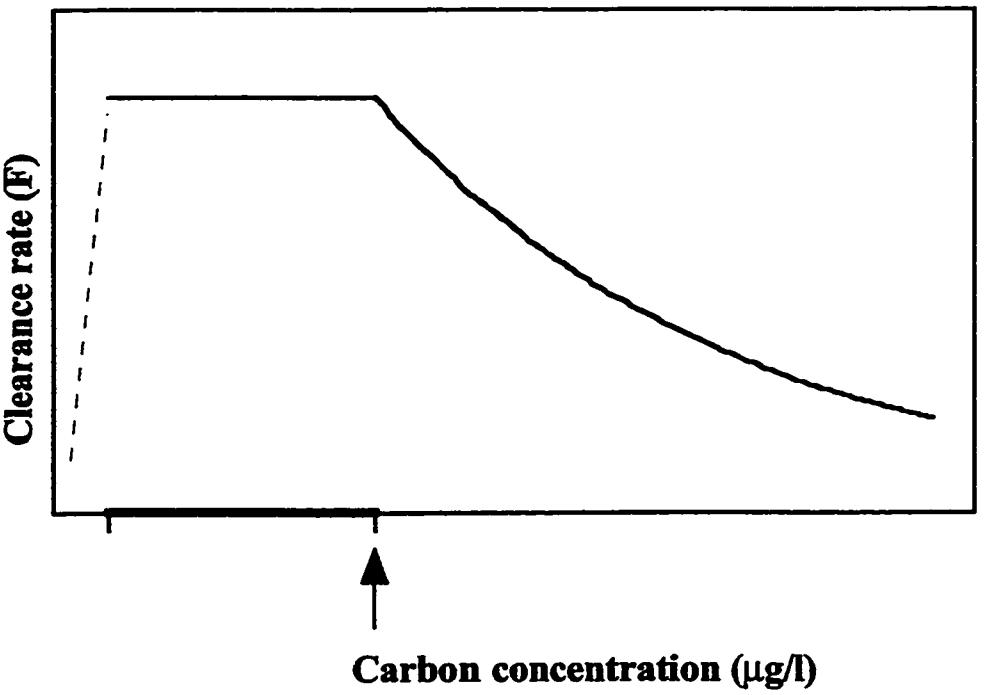
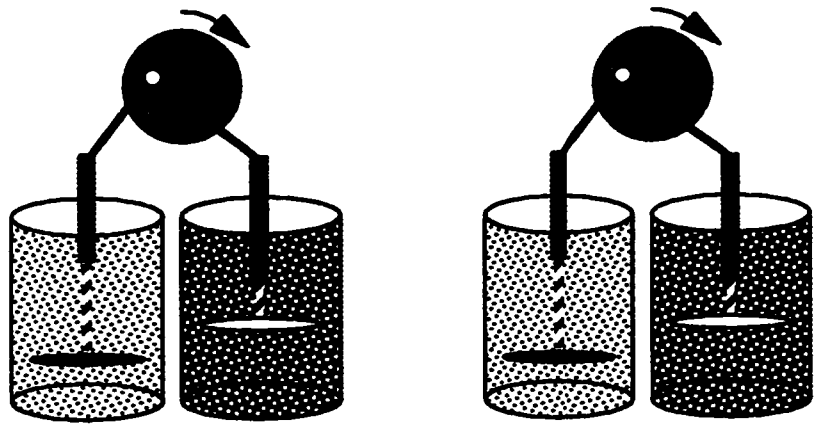

Clearance rate (F)
Carbon concentration (µg/l)

Fig. 2. **Experimental design and sampling protocol.**



TIME (h)	PAIR 1	PAIR 2
0-6	**CONTROL** (No krill) Water sampled at 0, 2, 4 and 6 h for chl a (5x100 ml) Water sampled and preserved at 0 and 6 h (1x100 ml)	**FEEDING PERIOD** (Krill in at 0 h) Water sampled at 0 h and 6 h for chl a
6-12	**FEEDING PERIOD 2** (Krill tranferred from Pair 2 to Pair 1) Water sampled at 8, 10 and 12 h for chl a Water sampled and preserved at 12 h (1x100 ml) Krill removed at 12 h Measured, weighed and frozen	**(NO ACTIVITY)**

Fig. 3. **Paired comparisons of clearance rates** ($l \bullet g$ wet $wt^{-1} \bullet h^{-1}$) between *Thalassiosira antarctica* (black bars) and small *Phaeocystis antarctica* colonies (gray bars) for:

a) single cell P. antarctica (1994-95)

b) small P. antarctica colonies (1993-94) and

c) medium P. antarctica colonies (1996-97). See Table 3 for statistical treatments.

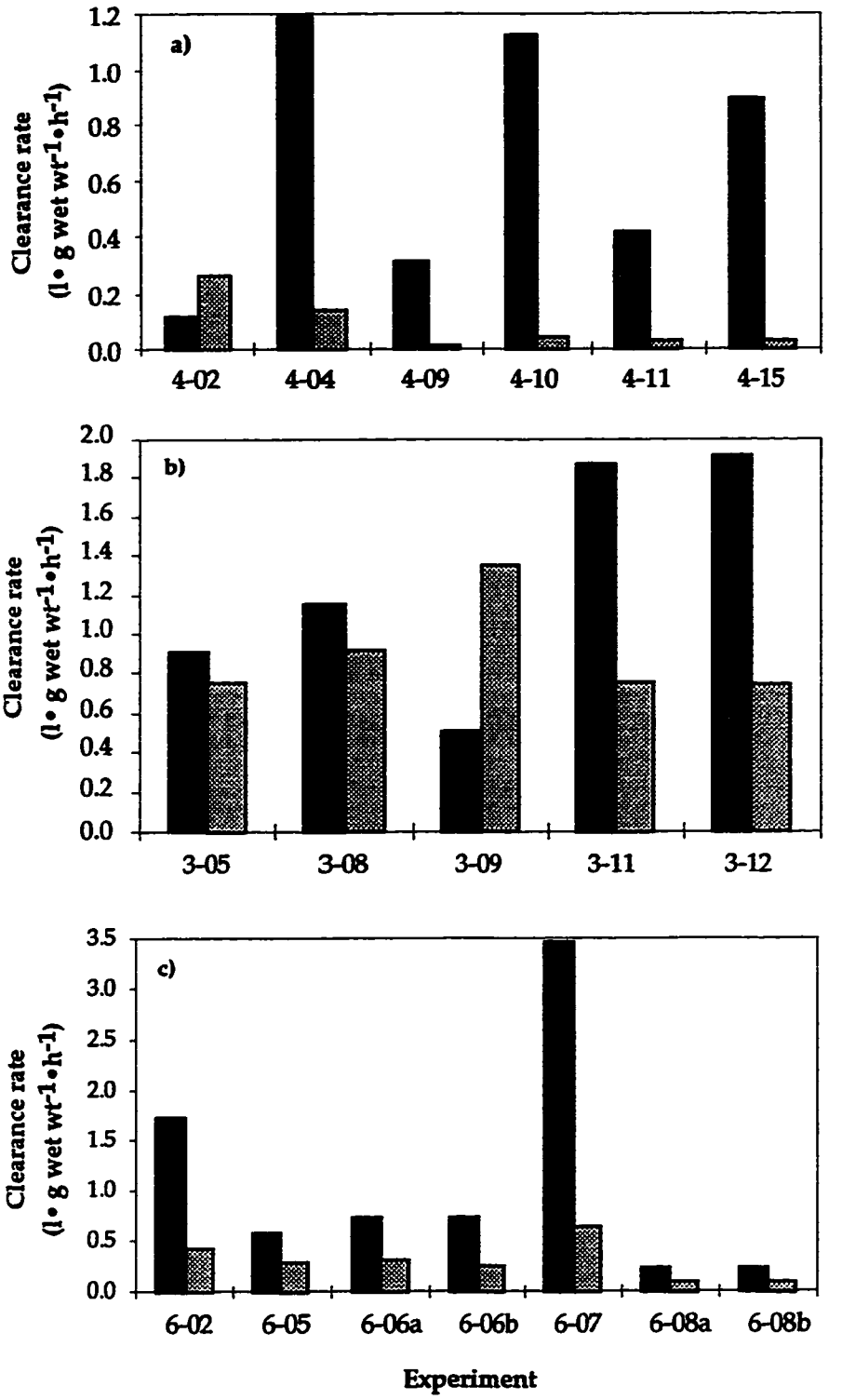

a)
Clearance rate
(l • g wet wt-1 • h-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
4-02
4-04
4-09
4-10
4-11
4-15
b)
Clearance rate
(l • g wet wt-1 • h-1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3-05
3-08
3-09
3-11
3-12
c)
Clearance rate
(l • g wet wt-1 • h-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
6-02
6-05
6-06a
6-06b
6-07
6-08a
6-08b
Experiment

Fig. 4. **Clearance rates (l•g wet wt^{-1}•h^{-1}) for *Euphausia superba* on *Thalassiosira antarctica* were plotted as a function of the percent of *T. antarctica* particles which were chains** of 2 or more cells for each individual years. OPEN SQUARES with SOLID regression line: 1993-94 ($y=2.8x-0.43$, $r^2=0.77$, $p=0.004$); OPEN DIAMONDS with DOTTED regression line: 1994-95 ($y=3.9x+0.44$, $r^2=0.20$, $p=0.13$); GRAY TRIANGLES with DASHED regression line: 1996-97 ($y=5.2x+0.34$, $r^2=0.75$, $p=0.02$).

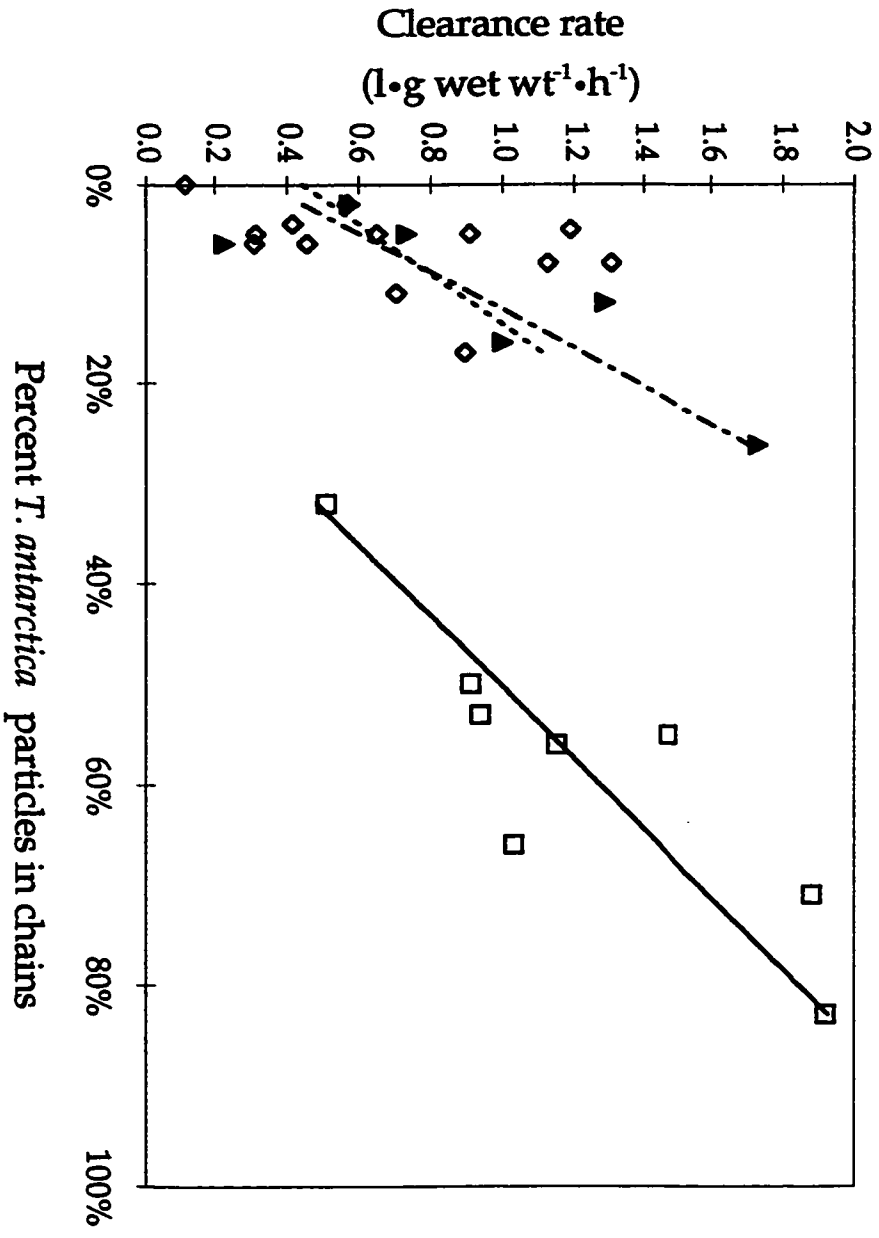

Clearance rate
(l•g wet wt^{-1}•h^{-1})
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0%
20%
40%
60%
80%
100%
Percent *T. antarctica* particles in chains

Fig. 5. **Clearance rates ($l \bullet g$ wet $wt^{-1} \bullet h^{-1}$) for *Euphausia superba* on *Phaeocystis antarctica* colonies were plotted as a function of initial colony size (μm)** using the median value of the maximum dimension (Table 1). $y=-0.004+1.11$,$r^2=0.72$, $p<0.001$.

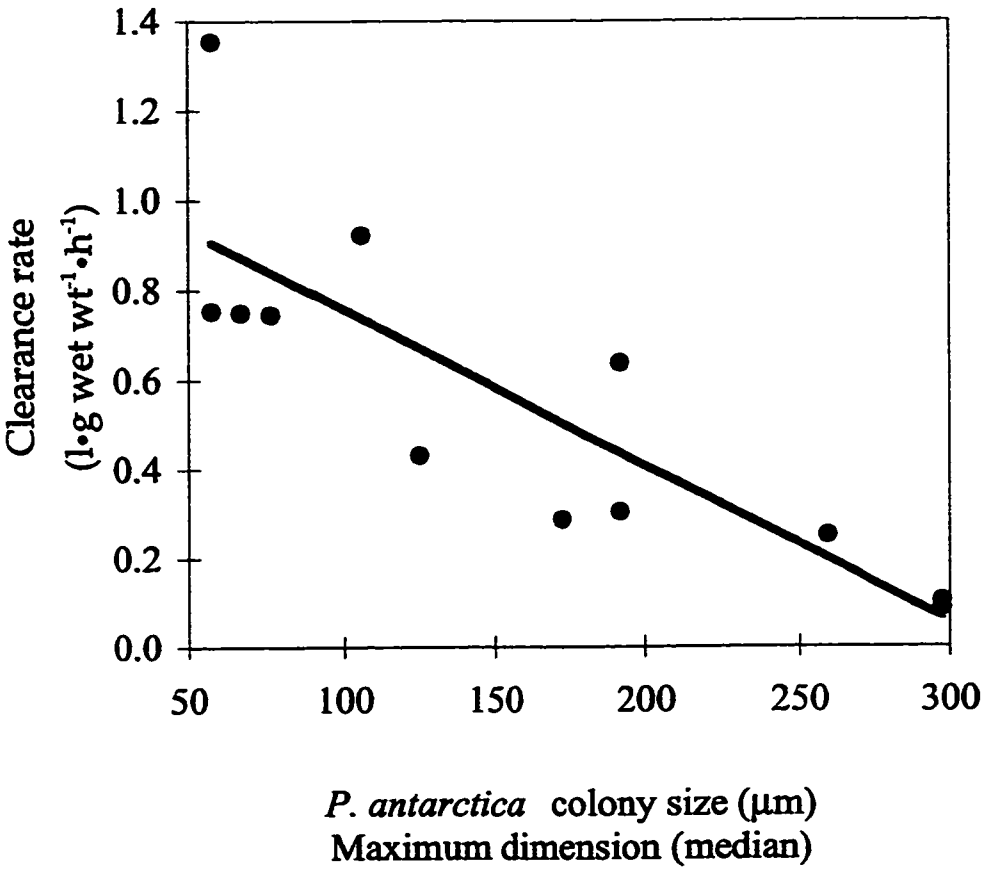

1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Clearance rate
(l•g wet wt-1•h-1)
50
100
150
200
250
300
P. antarctica colony size (µm)
Maximum dimension (median)

Fig. 6. **Ingestion rates of carbon and nitrogen by *Euphausia superba* grazing on *Thalassiosira antarctica*** for all years, plotted as a function of initial carbon concentration ($\mu g \bullet l^{-1}$). (OPEN SQUARES: 93-94, FILLED DIAMONDS:94-95, TRIANGLES: 96-97)

a) Regressions of carbon ingestion with initial carbon concentration among years were homogenous (p=0.99), and not significantly different from each other (ANCOVA, p=0.52). Overall regression (BLACK LINE): y=1.15x-42. r^2=0.67, p<0.001.

b) Regressions of nitrogen ingestion with initial carbon concentration among years were homogenous (p=0.45), and not significantly different from each other (ANCOVA, p=0.422). Overall regression (BLACK LINE): y=0.29x-15.8, r^2=0.63, p<0.001.

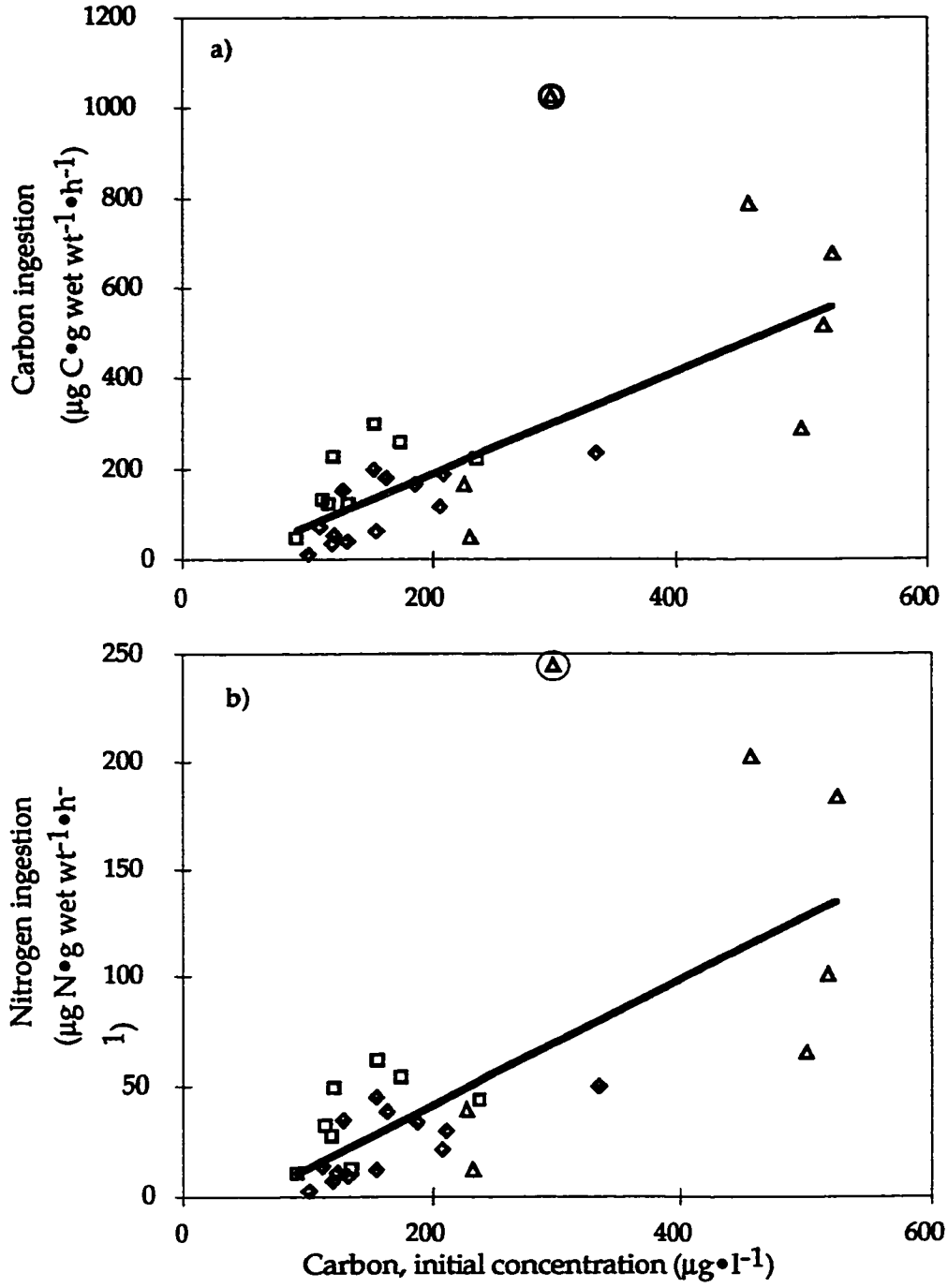
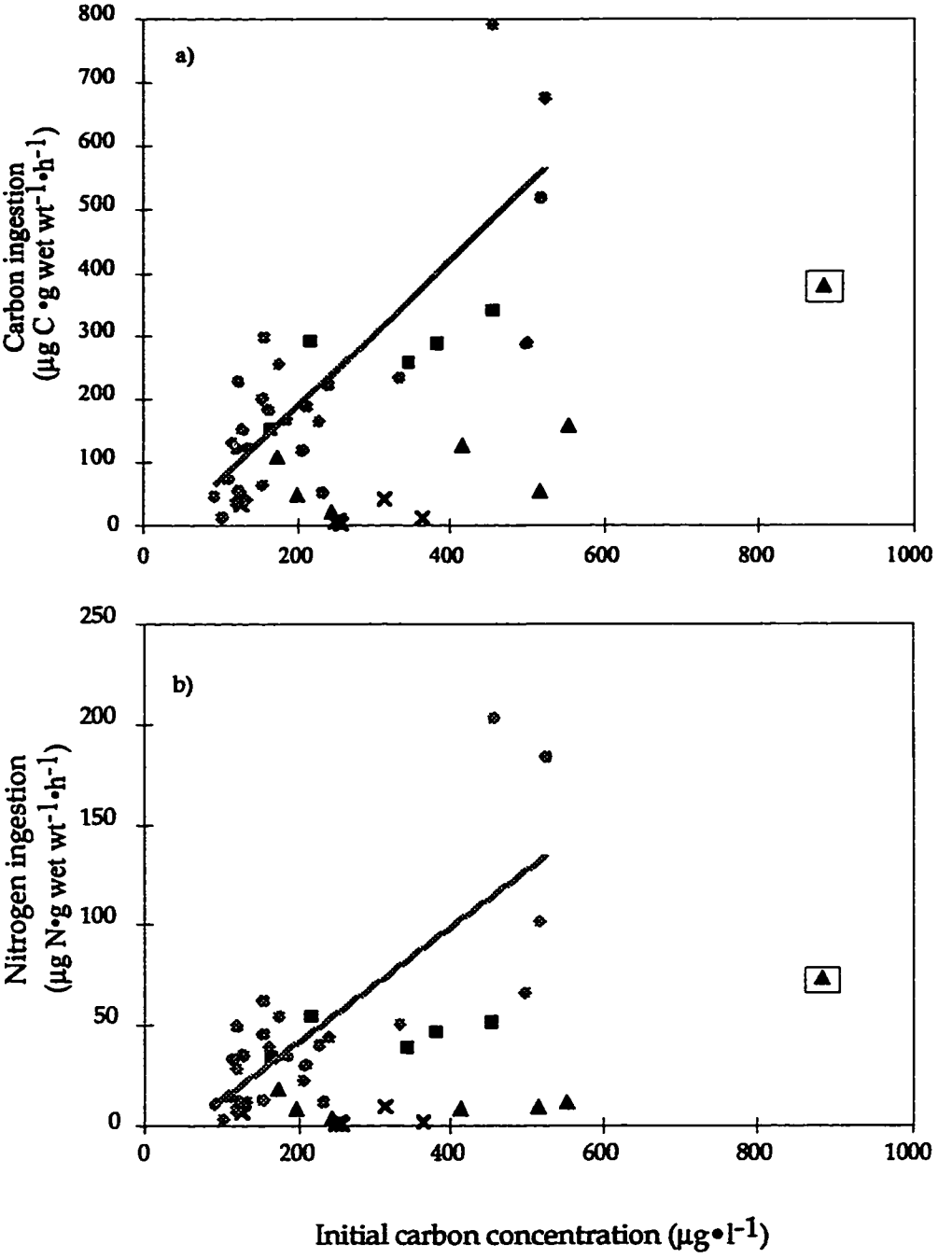

a)
Carbon ingestion
(µg C•g wet wt-1•h-1)
1200
1000
800
600
400
200
0
0
200
400
600
b)
Nitrogen ingestion
(µg N•g wet wt-1•h-
1)
250
200
150
100
50
0
0
200
400
600
Carbon, initial concentration (µg•l-1)

Fig. 7. **Ingestion rates of carbon and nitrogen by *Euphausia superba* grazing on *Phaeocystis antarctica*** for all years, plotted as a function of initial carbon concentration ($\mu g \bullet l^{-1}$). (FILLED SQUARES: sm col, 93-94; FILLED TRIANGLES: m col, 96-97; X: single cell, 94-95). Ingestion rates on *Thalassiosira antarctica* are included for comparison (GRAY CIRCLES). GRAY LINE indicates the regression for *T. antarctica*, inclusive for all years.

a) Regression of carbon ingestion with initial carbon concentration for was not significant for any year. The one outlier was excluded from analyses (see text).

b) Regression of nitrogen ingestion with initial carbon concentration was not significant for any year. The one outlier was excluded from analyses (see text).


a)
Carbon ingestion (µg C •g wet wt^{-1}•h^{-1})
800
700
600
500
400
300
200
100
0
0
200
400
600
800
1000
b)
Nitrogen ingestion (µg N•g wet wt^{-1}•h^{-1})
250
200
150
100
50
0
0
200
400
600
800
1000
Initial carbon concentration (µg•l^{-1})

CHAPTER 2

Assimilation of *Phaeocystis* -derived carbon and nitrogen by the Antarctic krill, *Euphausia superba*

ABSTRACT

Krill have been shown to ingest the prymnesiophyte *Phaeocystis antarctica*, although usually at lower rates than diatoms. However, the ability of the Antarctic krill to assimilate carbon and nitrogen from ingested *P. antarctica* has not been measured. In this study, ingestion and egestion rates of Antarctic krill fed *P. antarctica* were measured in the laboratory, and carbon and nitrogen assimilation efficiencies were calculated. Fecal pellets of krill fed *P. antarctica* did not readily sink and were easily fragmented, making fecal pellet collection difficult. Fecal pellets were collected in two ways.

The mean carbon assimilation efficiency of krill fed *P. antarctica* was 85%, and mean nitrogen assimilation efficiency was 94%. The C:N ratios of the fecal pellets, with a mean of 15.5, were significantly higher than the C:N ratios of the *P. antarctica* cultures, with a mean of 6.6. Assimilation efficiencies measured in this study were comparable to those for euphausiids grazing on other phytoplankton species. Since fecal pellets from krill fed *P. antarctica* tend to remain suspended and break apart, they probably do not contribute significantly to the vertical flux of either carbon or nitrogen.

INTRODUCTION

Antarctic krill, *Euphausia superba*, are capable of consuming the prymnesiophyte *Phaeocystis antarctica*, albeit at generally lower rates than they consume diatoms. Krill ingested small (50-100 μm diameter) *P. antarctica* colonies at rates similar to diatoms when they were presented alone (Chapter 1), but at lower rates than diatoms in mixed phytoplankton assemblages (Chapter 3). The dietary benefits of *P. antarctica* to the Antarctic krill depend not only upon the krill's ability to ingest *P. antarctica* in its various forms, but also upon the krill's ability to effectively assimilate nutrients from *P. antarctica*'s cells and colonies. Zooplankton digest and assimilate some phytoplankton species more effectively than others. For example, the cladoceran *Daphnia magna* was unable to assimilate most of the material available in colonies of the green alga *Sphaerocystis schroeteri*, which consist of cells embedded in a polysaccharide sheath. Ninety percent of the *S. schroeteri* cells remained viable after gut passage (Porter, 1976). Other species of "gelatinous" green algae were similarly resistant to digestion by cladocerans (Kerfoot et al., 1985). Likewise, the mucopolysaccharide matrix of colonial *P. antarctica* may prevent effective assimilation of

embedded cells. Even the non-motile, single cells of *P. antarctica* are encased in a transparent matrix, and could be difficult for zooplankers to digest.

In this study, we determined carbon and nitrogen assimilation efficiency of krill fed *Phaeocystis antarctica*. These assimilation efficiencies were calculated from rates of ingestion and egestion measured during laboratory grazing experiments.

METHODS

Egestion experiments. Egestion was measured by conducting fecal pellet production experiments immediately after grazing experiments on *Phaeocystis antarctica* (Chapter 1). Prior to the egestion experiment, four 4-l beakers and one 20-l bucket were filled with seawater filtered through a 0.45 μm filter and equilibrated at the experimental temperature (0.5 ±0.3 °C) for at least 6 hours. Beakers were numbered 1, 2, 3 and 4, and a mesh basket was placed in Beaker 1. The mesh basket was designed to separate krill and their fecal pellets, which generally sink to the bottom of the beaker.

At the end of the experimental feeding period (see Chapter 1), krill were removed from the experimental container, quickly rinsed in the 20-l bucket, and immediately transferred to the mesh basket in Beaker 1. At 15 minute intervals, krill were transferred to a new beaker by moving the mesh basket. Fecal pellet production was thus measured for the same group of animals for four consecutive 15 minute intervals. At the end of their 15 minute period in Beaker 4, krill were removed from the beaker, measured, weighed and frozen.

Collection of fecal pellets.

METHOD 1. For two assimilation efficiency experiments (3-05 and 3-11), individual fecal pellets were collected from the bottom of each 4-l beaker with a Pasteur pipet. Suspended pellets were also collected if observed. Pellets were rinsed with filtered seawater and examined with a dissecting microscope (6-50X magnification). Foreign material was removed with fine forceps. The samples, clean fecal pellets and filtered seawater (5-15 ml), were filtered onto pre-combusted (500 °C, 1 hour) Gelman A/E filters. An equivalent volume of 0.45 µm filtered seawater was filtered for a control. All filters were placed into pre-combusted (450 °C, 24 hours) aluminum sleeves and plates, stored in a

60 °C isotemp oven, and analyzed for carbon (C) and nitrogen (N) on a Leeman Laboratories AE 440 CHN Analyzer.

Unfortunately, fecal pellets produced during grazing on *Phaeocystis antarctica* tended to break apart and/or remain suspended in the beakers. In contrast, fecal pellets from diatom-fed krill remained intact and sank to the bottom. Fecal pellets produced in these experiments were also pale and difficult to see. As a consequence, the method of fecal pellet collection was modified for subsequent experiments.

METHOD 2. At the end of experiments 6-08 A, 6-10 A, and 6-10 B, the contents of each beaker were slowly poured into two 2-l jars, each with a funnel and 20-ml vial attached serially at the jar's mouth. The jar was inverted and floated in seawater tanks (0-1 °C) for two hours, allowing sinking fecal pellets to collect in the 20-ml vial. After carefully removing the 20-ml vial and pouring its contents into a petri dish, the remaining water was slowly poured through a 20 µm mesh sieve to remove suspended fecal material. The sieve's contents were rinsed with filtered seawater into a petri dish. Collected fecal pellets were then cleaned, filtered, stored and analyzed as described for the first collection technique. Control filters were obtained with 4-l beakers of filtered seawater.

Calculation of carbon and nitrogen egestion rates. Net carbon and nitrogen egested, $C_{E,net}$ and $N_{E,net}$, for each fifteen minute time period was calculated by subtracting the carbon or nitrogen mass, in μg, of the control filter from the carbon or nitrogen mass of the filter with collected fecal pellets. Egestion rates of carbon (E_C, in μg C g^{-1} h^{-1}) and nitrogen (E_N in μg N $g^{-1}h^{-1}$) were calculated from the first three time intervals only, since egestion rates in filtered seawater drop significantly after approximately 40 min (Clarke et al., 1988). Carbon and nitrogen egestion rates were calculated with the following equations:

$$(1)\ E_C = \sum_{1}^{x} C_{E,net} * (\text{wet weight in g})^{-1} * T^{-1}$$

$$(2)\ E_N = \sum_{1}^{x} N_{E,net} * (\text{wet weight in g})^{-1} * T^{-1}$$

In the above equation, x is the last fecal pellet collection beaker included (x=3 for all experiments) and T is the total time, in hours, for the fecal pellet collection periods used in the calculation. Wet weight refers to the total wet weight of the krill in the experiment.

Calculation of carbon and nitrogen ingestion rates. Ingestion rates for carbon, I_C (in $\mu g\ C\ g^{-1}\ h^{-1}$), and nitrogen, I_N (in $\mu g\ N\ g^{-1}\ h^{-1}$), were calculated as follows:

$$(3)\ I_C = F^*[\text{chl a}]_4\ ^*(\text{C:chl a})$$

$$(4)\ I_N = F^*[\text{chl a}]_4^*(\text{N:chl a})$$

F is the clearance rate for the experiment (see Chapter 1). C:chl a and N:chl a ratios were based on culture samples taken one day prior to the experiment (see Chapter 1). Chlorophyll a concentration ($[\text{chl a}]_4$) was based on water samples taken 4-h into the feeding period. Ingestion rates would have been overestimated if values from the beginning of the feeding period had been used, since a >14% drop in chlorophyll concentration occurred during the experiment (Marin et al., 1986).

Assimilation efficiency for carbon, AE_C, and assimilation efficiency for nitrogen, AE_N, were calculated as follows:

$$(5)\ AE_C = [1-(E_C/I_C)]^*100\%$$

$$(6)\ AE_N = [1-(E_N/I_N)]^*100\%$$

RESULTS

Experiments which used both gravity and sieving to collect fecal pellets yielded lower assimilation efficiencies than experiments which relied on picking out the fecal pellets directly from the 4-l beakers (Table 1). Although consistent, these differences were not statistically significant (Wilcoxon signed rank test, $p \leq 0.08$ for both carbon and nitrogen).

For krill fed *Phaeocystis antarctica*, mean carbon assimilation efficiency was 85±13%, and mean nitrogen assimilation efficiency was 94±5%. The difference between carbon and nitrogen assimilation efficiency was not significant (Wilcoxon signed ranked test, $p=0.07$). However, assimilation efficiency of nitrogen was more than 15% higher than carbon in two of the five experiments.

Significant differences were found between the C:N ratios of the fecal pellets and the *P. antarctica* cultures used as food in each experiment, which were 15.5±6.7(SD) and 6.6±0.6, respectively (Wilcoxon signed rank test, $p=0.04$). In two experiments, the C:N ratios of the fecal pellets were more than triple the C:N ratio of the corresponding *P. antarctica* culture.

DISCUSSION

In this study, Antarctic krill fed *Phaeocystis antarctica* assimilated carbon and nitrogen within the 72-94% range of efficiencies reported by Kato et al. (1982) for assimilation of other phytoplankton species by Antarctic krill. Our values here were also similar to those obtained by Ross (1982) for *Euphausia pacifica* grazing on the diatom *Thalassiosira* spp. Thus, the mucopolysaccharide matrix of the *P. antarctica* apparently did not affect digestion by the krill.

Although nitrogen assimilation was not significantly higher than carbon assimilation, the significantly higher C:N ratio of fecal pellets compared to phytoplankton cultures supports the conclusion that nitrogen was more efficiently assimilated by the krill. This result is consistent with other studies for zooplankton grazers (Landry et al., 1984; reviewed in Anderson, 1994), and with the observation that larval krill conserve nitrogen (Frazer, 1997). Higher assimilation of nitrogen may reflect the krill's nutritional requirements (Checkley, 1980; Cowles et al., 1988; Frazer, 1997) as well as the inability of zooplankton to digest structural carbohydrates, which are relatively high in carbon compared to nitrogen (Anderson, 1994).

Assimilation efficiencies calculated here may be overestimates. Both the ease with which the fecal pellets re-suspended and their fragile nature made quantitative collection difficult. Many of the fecal pellets were broken into tiny particles which were difficult to see even at 50X magnification. The constant and rapid motion of the krill in the collection beakers was sufficient to keep the fecal pellets in suspension as well as to break them into smaller particles. Most likely, fragments from some pellets passed through the 20 μm sieve. Some pellets may also have been re-ingested, although ingestion rates are relatively low in small containers (Price et al., 1988). In the future, shorter intervals in each beaker should be used to minimize the degree of fecal pellet break-up and the potential for re-ingestion.

Besides being difficult to collect, the fragile and slow-sinking fecal pellets produced by krill grazing on *Phaeocystis antarctica* are unlikely to contribute significantly to the vertical flux of either carbon or nitrogen to depth. Cadée et al. (1992) similarly found that fecal pellets produced by krill feeding on non-diatom phytoplankton species, including *Phaeocystis* sp., exhibited significantly slower sinking rates than fecal pellets produced by grazing on diatoms. Possibly, *P.*

antarctica-derived fecal pellets serve as an alternate food resource for other grazers within *P. antarctica* blooms.

CONCLUSION

A major proportion (>60%) of the organic carbon and nitrogen ingested during grazing by the Antarctic krill on *Phaeocystis antarctica* was initially assimilated. Krill generally assimilated nitrogen more efficiently than carbon, resulting in fecal pellets with significantly higher C:N ratios than the *P. antarctica* cultures on which the krill grazed. The fragile nature and relatively high buoyancy of fecal pellets derived from *P. antarctica* suggest that their contribution to sinking flux of carbon and nitrogen is minimal compared to fecal pellets derived from diatoms.

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Table 1. Assimilation efficiency and C:N ratios of fecal pellets for all fecal pellet production (FPP) experiments. FPP method is the collection method used for the fecal pellets. See text for method descriptions. Abbreviations: sm col= small colonies; lg col =large colonies, single=single cells.

Experiment	*P. antarctica* Description	FPP Method	Assimilation efficiency Carbon	Assimilation efficiency Nitrogen	C:N ratio Food	C:N ratio Fecal pellets
3-05	sm col	1	93%	98%	6.6	24
3-11	sm col	1	97%	98%	6.2	9.3
6-08 A	lg col	2	79%	95%	5.8	19
6-10 A	lg col, single	2	93%	93%	7.2	8.3
6-10 B	lg col, single	2	65%	86%	7.2	17
MEAN			**85%**	**94%**	**6.6**	**15.5**
SD			13%	5%	0.6	6.7

CHAPTER 3

Selective grazing by the Antarctic krill, *Euphausia superba*, in mixed phytoplankton assemblages

ABSTRACT

Phytoplankton is a primary food resource for Antarctic krill. However, the relative contribution of different phytoplankton groups to the diet of krill is unknown. Prymnesiophytes (i.e. *Phaeocystis*) and cryptophytes can also comprise a significant proportion of the phytoplankton community. All these phytoplankton groups could be utilized by the krill. In this study, selectivity for different phytoplankton taxa by *Euphausia superba* was examined during laboratory grazing experiments with mixed phytoplankton assemblages from the wild and from laboratory cultures. Clearance rates and changes in relative concentrations of diatoms, prymnesiophytes and cryptophytes were measured by analysis of taxon-specific accessory photopigments with high pressure liquid chromatography (HPLC). *E. superba* grazed diatoms at higher rates than *Phaeocystis* and cryptophytes. Grazing was negligible in cryptophyte-dominated assemblages. Increases in 19'-hexanoyloxyfucoxanthin:fucoxanthin (prymnesiophyte:diatom) ratios up to 369% indicated a high level of selectivity for diatoms over *Phaeocystis*. This selectivity occurred even for *Phaeocystis* colonies of

similar size to diatoms, thus it cannot be entirely attributed to differential sieving efficiency based on particle size. The results of these experiments suggest that krill actively select diatoms in phytoplankton mixtures.

INTRODUCTION

Antarctic krill, *Euphausia superba*, graze phytoplankton of different sizes with different efficiencies. Several studies have shown that krill filter larger particles, up to at least 50 μm equivalent spherical diameter, at higher rates than smaller particles (McClatchie and Boyd, 1983; Boyd et al., 1984; Quetin and Ross, 1985; Ishii et al., 1985). Euphausiids may also graze diatoms in chains more effectively than solitary cells (Chapter 1; Meyer and El-Sayed, 1983; Stuart, 1989) but this criterion for selection is difficult to separate from size. Previously, observed selectivity has been linked to size of the cell and efficiency of the sieving apparatus (McClatchie and Boyd, 1983), and not to an active process. Here we ask, do krill select particular phytoplankton types based on characteristics other than size?

Other species of filter-feeding zooplankters, copepods in particular, show remarkable discrimination with regard to food quality. Some copepods appear to forage optimally, preferentially ingesting more nutritional cells within mixtures (Cowles, 1988) and rejecting low quality particles such as polystyrene spheres, dead phytoplankton, and phytoplankton species resistant to digestion (Donaghay and Small,

1979; Paffenhöfer and Van Sant, 1985; DeMott, 1989), as well as toxic species (Huntley et al., 1986).

Phytoplankters are often grouped and quantified taxonomically. The relative abundances of these different phytoplankton taxa may be relevant to the feeding ecology of zooplankton grazers. For example, several species of filter-feeding copepods have demonstrated taxon-specific selectivity. In one study, several species of copepods ingested diatoms at higher rates than prymnesiophytes within natural phytoplankton assemblages (Head and Harris, 1994), while in another, dinoflagellates were avoided (Quiblier-Llobéras et al., 1996). *Acartia* spp. selected diatoms over *Phaeocystis* in laboratory grazing experiments, although this result may have been a consequence of particle size (Verity and Smayda, 1989).

The key taxonomic groups available to Antarctic krill in the coastal regions of the Antarctic Peninsula are diatoms, prymnesiophytes and cryptophytes. Although one group may periodically dominate, they also co-exist (Moline and Prézelin, 1996). At the Weddell Sea ice edge, the highest concentrations of both diatoms and *Phaeocystis* were generally in mixtures of the two groups (Fryxell and Kendrick 1988). Currently, it is unknown whether Antarctic krill are selective for one

or more of these key taxonomic groups. If only size selection occurs, krill would graze the cryptophytes less effectively than diatoms, since they are smaller. On the other hand, high cryptophyte concentrations could compensate for lower filtration efficiency. With regard to prymnesiophytes, Haberman (Chapter 1) showed that clearance rates on colonies of the prymnesiophyte *Phaeocystis antarctica* and the diatom *Thalassiosira antarctica* were similar when both species offered as unialgal cultures in the laboratory, whereas single cells and larger colonies were grazed at lower rates. However, Haberman (Chapter 1) did not address selectivity between the two taxonomic groups in mixed assemblages.

Feeding mechanisms differ between krill and copepods. Specifically, krill capture many particles at once with their mechanism of compression-filtration (Hamner, 1988), while copepods tend to actively capture individual particles (Price and Paffenhöfer, 1985). Also, euphausiids are considerably larger than copepods. Thus, selectivity in euphausiids will not necessarily follow that in copepods. Only one study of a euphausiid (Stuart, 1989) has examined selective feeding on a criterion other than size. Stuart (1989) found that *Euphausia lucens* selected dinoflagellates in preference to diatoms in

field-collected phytoplankton mixtures, despite the smaller size of the dinoflagellates and the high abundance of diatoms. This study was designed to test whether *Euphausia superba* selectively graze one or more of the key taxonomic groups in the Antarctic Peninsula region. Grazing by *Euphausia superba* on the different phytoplankton groups during laboratory experiments was measured using taxon-specific accessory photopigments to track the phytoplankton levels in the experimental containers. Krill were presented with both mixtures of phytoplankton cultures as well as phytoplankton assemblages collected from the field.

METHODS

Collection and maintenance of krill and phytoplankton. Krill were collected from either a zodiac or the M/V Polar Duke, and maintained in 2.5-m diameter flow-through seawater tanks at Palmer Station. Details of collection, maintenance, and selection of healthy animals for experiments were described previously (Chapter 1).

Phytoplankton for experiments with mixtures of unialgal cultures were grown at 1.5-2.0 °C in 20-l clearboy containers aerated by Silent Giant pumps with sterile filtration (Gelman bacterial airvents). Cultures were illuminated by cool white fluorescent lights on a cycle of 18 hours on, 6 hours off. *Thalassiosira antarctica* and *Phaeocystis antarctica* were both isolated from the Palmer Station region (*P. antarctica* isolated by Dr. D Karentz, *T. antarctica* isolated by the author). Cultures were usually maintained in f/2 media (Guillard and Ryther, 1962). However, the media for *P. antarctica* did not contain silica, and in some cases the media strength was altered (Chapter 1). Culture growth was assessed bi-weekly using chlorophyll a (chl a) analysis (Chapter 1). Cultures were also evaluated microscopically to measure cell, chain and colony size, and to check for contamination.

Water samples for experiments with natural phytoplankton assemblages were collected within two miles of Palmer Station. For some experiments, water was collected with Go-Flo bottles deployed from a zodiac to the chlorophyll maximum layer, as determined by a vertical profile of *in situ* fluorescence. Immediately following collection, these water samples were transferred to containers for acclimation to experimental conditions. Alternately, water was collected from the unfiltered seawater intake at Palmer Station.

Within 24 hours prior to the experiment, cultures or collected phytoplankton were sampled for chl a and elemental (CHN) analysis, and 100 ml subsamples were preserved in 0.35% glutaraldehyde/Lugol's solution (Rousseau et. al., 1990) for additional microscopic evaluation. (Details of subsampling and evaluation are described in this volume, Chapter 1). Chl a, C and N concentrations from these analyses were used to calculate ratios of C:N and C:chl a. Selected cultures were also analyzed for pigment content using high performance liquid chromatography (HPLC), as described later in this section.

Experimental set-up and sampling. Two identical containers were prepared for each experiment, one for acclimation of krill to feeding conditions, and the other for the measurement of control and experimental grazing rates. Experimental containers were 47 cm in diameter and 53 cm in height and were filled with 55-60 liters of phytoplankton, which was mixed continuously with a plunger-type stirrer system modified after that of Frost (1972). Four duplicate experiments were conducted with field-collected phytoplankton at ambient concentrations. Six additional experiments were conducted with mixtures of *Thalassiosira antarctica* and *Phaeocystis antarctica* cultures in approximately equal concentrations of chl a, and diluted with 0.45 μm filtered seawater to final chl a concentrations ranging from 3-18 $\mu g \cdot l^{-1}$. Phytoplankton was added to the containers several hours before the experiments in order for the mixture to equilibrate to experimental temperature and light conditions (0.5 ±0.3 °C, dim light). Krill were also kept in experimental conditions in 18-l buckets of filtered seawater during this equilibration period. Ten to thirty krill, ranging in size from 34-42 mm, were utilized in each experiment.

Most experiments lasted twelve hours. During the first six hours, krill were acclimated to experimental food conditions in one of the

replicate containers with food. Krill were then transferred from the acclimation container to the experimental container, where they grazed for an additional six hours (Chapter 1, Fig. 2). Three 100-ml water samples were collected from the acclimation container at the beginning and end of the acclimation period. Five 200-ml (or three 350-ml) water samples were collected from the experimental container at 2-h intervals throughout the 12-h experiment. Samples from the experimental container for the first 6-h period without krill ("control period") were used to calculate the rate of change of phytoplankton pigments without grazing, while samples from the second 6-h period ("experimental period") were used to calculate change in pigment concentrations due to both growth and grazing.

For two of the six *Thalassiosira antarctica*:*Phaeocystis antarctica* mixture experiments, the krill were not acclimated to experimental feeding conditions and control rates were not measured (Table 2). The control rate for pigment loss and ratio change for these experiments was assumed to be 0, since no significant changes occurred during the control period for any of the other experiments with the same phytoplankton cultures (see Results).

Water samples were filtered immediately after collection. One hundred to two-hundred and fifty milliliters of each sample was filtered onto 0.45 μm nylon filters for HPLC, while the remaining 100 ml of each sample was filtered onto GF/C filters for fluorometry. Filters were frozen at -70 °C and analyzed within 4 weeks of collection.

Pigment analysis. Pigments from experimental samples and selected phytoplankton cultures were analyzed with high performance liquid chromatography (HPLC) using a method modified from Wright et al. (1991). The system consisted of an Hitachi Instruments L-6200 A pump and gradient controller, L-4250 UV/VIS detector set at 436 nm, and D-6000 software connected via an Hitachi interface. A Waters Resolve C-18 5μm, 3.9x300mm column was used for pigment separation. All solvents were HPLC grade, and included:

Solvent A: 80:20 (v:v) Methanol: 0.5 M ammonium acetate

Solvent B: 90:10 (v:v) Acetonitrile: water

Solvent C: Ethyl acetate

The solvent gradient protocol is shown in Table 1. Flow rates were constant at 1.0 ml/min.

Samples were extracted in 3-5 ml (depending upon sample concentration) of 90% HPLC grade acetone for 24 h at 0 °C. Samples were then centrifuged to remove debris, and the supernatant used for pigment analysis. Extracted samples were kept at 0°C prior to analysis.

To identify and quantify sample pigments, columns were calibrated with commercially obtained pigment standards of known concentrations (VKI Water Quality Institute, Hørsholm, Denmark) and with chl a derived from *Anacystis* sp. (Sigma). The key accessory photopigments present in field-collected water samples and used to evaluate grazing selectivity were fucoxanthin (FUCO) as a diagnostic pigment for diatoms, 19'-hexanoyloxyfucoxanthin (HF) as a diagnostic pigment for prymnesiophytes, and alloxanthin (ALLO) as a diagnostic pigment for cryptophytes (Wright et al., 1991).

Chl a concentrations were also determined by fluorometry (Smith et al. 1981) and clearance rates calculated from the chl a concentrations (Table 2), so that these values could be directly compared with similar experiments analyzed only by fluorometry (Chapter 1).

Clearance rate calculations. Clearance rates for each key pigment type (chl a, FUCO, HF and ALLO) were calculated based on the changes

in pigment concentrations during both the experimental and control periods. The phytoplankton loss coefficient (b) was calculated from the experimental period with the best fit of the exponential function:

$$(1)\ [\text{pigment}]_t=[\text{pigment}]_0 * e^{bt}$$

The phytoplankton growth coefficient (k) was calculated from the control period with the best fit of the the exponential function:

$$(2)\ [\text{pigment}]_t=[\text{pigment}]_0 * e^{kt}$$

The grazing coefficient, g (Frost, 1972) was calculated as:

$$(3)\ g = k-b$$

Pigment-specific clearance rate ($F=l \bullet g$ wet $wt^{-1} \bullet h^{-1}$), i.e. the wet-weight specific rate at which a given volume of seawater is swept clear of the particular pigment (after Frost, 1972) was calculated from g, the volume of the phytoplankton mixture in liters (V), and the wet weight of krill per container (W).

$$(4)\ F=g*V/W$$

Pigment ratio calculations. Pigment ratios were calculated from individual chromatograms to minimize variability due to injection volume, and then averaged for each time period. In addition, percent change in these ratios from the beginning (Time 6) and end (Time 12) of the experimental period were used to determine the magnitude of selectivity for experiments with signficant changes in pigment ratios. Percent ratio change was calculated as:

(5) % ratio change=100%*(ratio,T12-ratio,T6)/ratio,T6

Ratio changes during control periods were not signficant (see results), so they were not included in this calculation.

RESULTS

Grazing on mixtures of *Phaeocystis antarctica* and *Thalassiosira antarctica* cultures.

Characterization of cultures. Particle sizes for *Phaeocystis antarctica* and *Thalassiosira antarctica* were similar. The *P. antarctica* cultures used in these experiments were comprised of small spherical or elliptical colonies between 50-100 μm, with <20% single cells. *T. antarctica* was comprised of cells 25 μm in diameter, occurring singly and in chains predominantly 2-4 cells and 30-90 μm in length. C:N values for the two cultures were similar, 5.3±1.1 (SD) for *P. antarctica* and 5.5±1.7 for *T. antarctica.* C:chl a for *P. antarctica* was roughly double that of *T. antarctica,* 60±18 compared to 30±6. Initial chl a concentrations were 3-18 $\mu g \cdot l^{-1}$.

The HF:FUCO ratios for the 1:1 mixtures (by chl a) of *Phaeocystis antarctica* and *Thalassiosira antarctica* cultures ranged between 0.3 and 0.7 for the six experiments (Table 2). The *P. antarctica* cultures used in the mixture experiments had FUCO:chl a ratios of 0-0.07, compared to 0.64-0.69 for the *Thalassiosira antarctica* cultures used in experiments

(Table 3). This means that most (≥90%) of the fucoxanthin in the mixtures was derived from *T. antarctica,* and supports the use of fucoxanthin concentrations as a proxy for diatoms in these experiments.

Clearance rates. *Euphausia superba* measurably grazed phytoplankton in all experiments, and chl a clearance rates were 0.45-1.87 $l \cdot g\ wet\ wt^{-1} \cdot h^{-1}$ (Table 2). Fucoxanthin clearance rates were similar to, or higher than, those for chl a. In contrast, 19'-hexanoyloxyfucoxanthin clearance rates were always lower than for chl a, and ranged between 0-51% of fucoxanthin clearance rates, indicating selectivity for diatoms in all experiments.

Overall, chl a clearance rates varied considerably among experiments, but there were no obvious correlations between clearance rates and other factors. This variability was not correlated with initial chl a concentration (regression, r^2=0.06), or the initial HF:FUCO ratio (r^2=0.25, p=0.32). Furthermore, there was a large difference in clearance rates between experiments 3-07 A and 3-07 B, which were conducted simultaneously and used nearly identical phytoplankton mixtures and krill.

Pigment ratio changes. Coincident with the differences in clearance rates for the two accessory photopigments, the HF:FUCO ratios increased significantly during all experiments compared to controls, with increases of 11-369% (Fig. 1, Table 2). There were no significant ratio changes in any of the control containers. Experiments with the highest clearance rates showed the largest changes in pigment ratios, with the percent change in the HF:FUCO ratio an exponential function of the clearance rate (Fig. 2).

Grazing on natural phytoplankton assemblages

Characterization of phytoplankton assemblages. The eight grazing experiments with field-collected phytoplankton assemblages were grouped based upon initial ratios of ALLO:HF:FUCO (Table 2). The phytoplankton concentrations were assumed to be roughly equivalent to the corresponding accessory pigment concentrations. In two experiments, the three pigments were present in approximately equal concentrations, indicating similar amounts of cryptophytes, *Phaeocystis* and diatoms. In four other experiments, alloxanthin

concentrations were more than double those of 19-hexanoyloxyfucoxanthin and fucoxanthin, indicating that cryptophytes were relatively abundant compared to diatoms and *Phaeocystis*. In the two remaining experiments, no alloxanthin was detected, and 19'-hexanoyloxyfucoxanthin and fucoxanthin were present in approximately equal concentrations.

For the six cryptophyte-containing experiments, initial chl a concentrations were between 3-7 $\mu g \cdot l^{-1}$, and C:chl a ratios were between 66-109. C:N ratios were similar, with a mean value of 5.3±0.6. The two experiments without cryptophytes had C:N ratios of 10.43, nearly double the values of other experiments, and high C:Chl a ratios of 367, 3-6 times the values of the other natural assemblage experiments.

Clearance rates and pigment ratio changes. In the two experiments with approximately equal concentrations of the three phytoplankton groups, significant clearance of chl a was measured, 0.53 and 0.15 $l \cdot g$ wet $wt^{-1} \cdot h^{-1}$, respectively. In addition, clearance rates for all three accessory pigments were significantly different from zero; however, rates for fucoxanthin were higher by at least 50% than rates for 19'-

hexanoyloxy-fucoxanthin or alloxanthin (Table 2). In addition, the HF:FUCO ratios increased by 65% and 31%, respectively, in the two experiments (Fig. 3, Table 2). In one of these two experiments, a large and significant increase (118%) in the ALLO:FUCO ratio also was measured (Fig. 4a, Table 2). The ALLO:FUCO ratio changes for the other experiment were difficult to evaluate due to the large error measurements (Fig. 4b). Overall, the differential clearance rates and pigment ratio changes indicated that *Euphausia superba* selectively grazed on diatoms in these experiments.

For the phytoplankton assemblages with relatively high concentrations of cryptophytes compared to diatoms and *Phaeocystis* sp., the clearance rates on chl a equaled zero in three of the four experiments, and the clearance rate was only 0.05 $l \cdot g$ wet $wt^{-1} \cdot h^{-1}$ in the remaining experiment (Table 2). However, in two of these experiments, there was a significant increase in the HF:FUCO ratios of approximately 9% (Fig. 5), as well as a relatively low but measurable clearance rate for fucoxanthin (Table 2). These results indicated that *Euphausia superba* selectively grazed on diatoms, but this grazing had no effect on the overall chl a concentration because diatoms were such a minor component of the assemblage.

Very low chl a clearance rates were measured for the two field-collected phytoplankton experiments with diatoms and prymnesiophytes only (Table 2). This assemblage was considerably lower in chl a (Table 2) and potentially much higher in detritus, based on their high C:N and C:chl a ratios. No pigment ratio changes were detected.

DISCUSSION

Methodological considerations. The use of accessory photopigments to study taxon-specific grazing on phytoplankton was first applied to microzooplankton grazers (Burkill et al., 1987), but has more recently been used in studies of macrozooplankton grazers (Head and Harris, 1994; Quiblier-Llobéras, 1996; Buffan-Dubau, 1996). This approach is based on two major assumptions: 1) Each accessory photopigment is derived primarily from one taxon and thus represents only the concentration of that taxon and 2) changes in accessory photopigment concentrations in experimental containers are not confounded by pigments in fecal material. Additionally, in this study, accessory photopigment values for natural phytoplankton assemblages were not converted to absolute concentrations of phytoplankton taxa, based on the assumption that: 3) pigment ratios roughly mirror relative concentrations of phytoplankton taxa. Here, we examine the validity of these assumptions.

The first assumption, that accessory photopigments are taxon-specific, is not strictly accurate. In particular, the diagnostic pigment for diatoms, fucoxanthin, is also found in varying concentrations in

prymnesiophytes (Wright et al., 1991). Fortuitously, colonial *P. antarctica* cultures used in these mixture experiments contained very low concentrations of fucoxanthin. However, Buma et al. (1991) found significant concentrations of fucoxanthin in cultured *Phaeocystis* sp. from the Weddell Sea, especially in single cells, as also found by Haberman (Appendix 1). The effects of *Phaeocystis*-derived fucoxanthin should be considered when interpreting the results of natural phytoplankton experiments. With significant percentages of fucoxanthin derived from *P. antarctica*, selectivity for diatoms would be underestimated by HF:FUCO ratio changes, since there would be less of a percent change in the fucoxanthin as diatoms were grazed. So, HF:FUCO ratio changes reported in this study are conservative estimates of selection by krill for diatoms.

The second assumption, that changes in pigment concentrations are not confounded by pigments in fecal material, is supported in two ways. First, large experimental containers were used, resulting in low krill density (≤0.5 krill/l). Consequently, water samples contained very little fecal material. Even at the end of the experiment, when fecal material had accumulated in the containers, there were few fecal pellets per sample, probably too few to affect pigment concentrations.

Second, based on FUCO:chl a ratios in *Euphausia superba* fecal pellets and the degree of chl a degradation by*E. superba* during gut passage (Daly, 1998), most of the fucoxanthin ingested by krill is degraded during gut passage. Even if not completely degraded, fucoxanthin and 19'-hexanoyloxyfucoxanthin are structurally similar and would be degraded by similar mechanisms (Nelson, 1989; Head and Harris, 1992). Thus, their relative levels of destruction should be similar, and the observed differences in pigment loss most likely reflect grazing differences, rather than differential pigment degradation.

The third assumption, that pigment ratios roughly mirrored phytoplankton concentrations, was examined for both cultured and field-collected phytoplankton. Cultures of *Phaeocystis antarctica* and *Thalassiosira antarctica*, mixed in 1:1 proportions on the basis of chl a concentration, had a mean HF:FUCO ratio of 0.4±0.1. In this case, direct conversion of pigment ratios into phytoplankton proportions would overestimate the proportion of diatoms by 100%. However, HF:FUC ratios of 1.25-1.30 for equal *Phaeocystis* :diatom mixtures were calculated from chl a:HF and chl a:FUC ratios determined by multiple regression of accessory photopigments versus chl a in field-collected phytoplankton (Bidigare et al., 1996; M. Vernet, personal

communication), Thus, HF:FUCO ratios unadjusted for differences in pigment:chl a ratios adequately approximate the actual proportions of *Phaeocystis* and diatoms. For cryptophytes and diatoms, ALLO:FUCO ratios of 0.4-0.6 were calculated for equal mixtures of cryptophytes and diatoms, based on multiple regression equations for field collected phytoplankton (Moline and Prézelin, 1996; M. Vernet, personal communication). These values suggest that the cryptophyte:diatom ratio is approximately double the ALLO:FUCO ratio. Thus, assemblages with high proportions of alloxanthin were dominated by cryptophytes to an even greater extent than shown by this analysis, making selectivity for diatoms more striking.

Grazing selectivity by *Euphausia superba*. *Euphausia superba* exhibited three distinct patterns in grazing during this study. First, *E. superba* selected diatoms over *Phaeocystis antarctica* in both culture mixtures and in most natural phytoplankton assemblages. Second, based on clearance rates, *E. superba* usually selected diatoms over cryptophytes. However, high levels of alloxanthin variability between subsamples, along with relatively high concentrations of alloxanthin, made small changes in ALLO:FUCO ratios difficult to detect. Third,

negligible grazing occurred on phytoplankton assemblages dominated by cryptophytes, even when chl a concentrations were above 6 $\mu g \cdot l^{-1}$.

In two of the three experiments with the highest percent change in ratio (3-07 B and 3-10), selectivity apparently remained constant as diatoms became proportionally more scarce through time. This inference is based on the exponential relationship between HF:FUC ratios and time found for these experiments. Mathematically, a positive exponential curve, as seen in these experiments, is predicted for HF:FUC over time if the phytoplankton loss coefficients for each phytoplankton type (b_{FUC} and b_{HF}, following Equation 1) are constant through time, and $b_{FUC} < b_{HF}$ (both coefficients are negative). This curve is described by the equation:

$$(6)\ (HF/FUC)_t=(HF/FUC)_0 e^{(b_{HF}-b_{FUC})_t}$$

In a similar manner, the exponential relationship between percent change in HF:FUC ratio and clearance rate for experiments on culture mixtures (Fig. 2) is predicted mathematically if selectivity on diatoms is independent of the relative concentration of diatoms in the mixture.

In contrast, optimal foraging theory predicts that selectivity will decline along when the high-quality food becomes scarce (MacArthur and Piantka, 1966; Lehman, 1976), as found for the copepods *Eudiaptomus* spp. (DeMott, 1989). Possibly, diatom density was never low enough in the mixed culture experiments to cause a shift in selectivity.

Particle size differences may account for selection of diatoms over cryptophytes. During experiments 3-03 A and B, krill cleared cryptophytes at approximately 50% of their rate on diatoms, similar to size-based selectivity previously described for 8-10 μm diameter particles compared to 30-50 μm particles (Boyd et al., 1984; Quetin and Ross, 1985; McClatchie and Boyd, 1983). However, the complete lack of grazing on cryptophytes when cryptophytes were the primary taxon cannot be explained by retention efficiency alone, since krill consumed cryptophytes in the more equal mixtures.

Size differences were probably also a factor iin the selectivity of diatoms over *Phaeocystis* in the natural phytoplankton experiments, since the *Phaeocystis* was a mixture of colonies and single cells approximately 8 μm in diameter, similar to the size of cryptophytes (Haberman, personal observation). However, size alone would not

explain consistent selection of *T. antarctica* over *P. antarctica* by krill in the culture mixtures. In those experiments, *T. antarctica* and *P. antarctica* were approximately the same size, based on both ranges and median values of maximum particle dimension (Chapter 1). Furthermore, monoculture experiments showed that krill were capable of clearing small colonies of *P. antarctica*, identical to those used in these experiments, at rates similar to *T. antarctica* (Chapter 1). Thus, in mixtures, krill must have either actively avoided filtering *P. antarctica* or rejected *P. antarctica* after filtration. Particle rejection is more likely in this situation, because the phytoplankton was well mixed in the experimental containers.

Size alone cannot account for observed patterns of selection between *Thalassiosira antarctica* and colonial *Phaeocystis antarctica*; thus, other aspects of food quality must be considered. In a recent study, growth rates of juvenile *Euphausia superba* (15-25 mm) were negatively correlated with percent *Phaeocystis* in the phytoplankton community (Ross et al., submitted). In general, *Phaeocystis* has been considered a sub-optimal food resource for macrozooplankton grazers (reviewed by Davidson and Marchant, 1992). Much of *Phaeocystis*' reputation as a poor food resource is based on indirect evidence,

including multiple studies which show that many copepod species and the euphausiid *Thysanoessa* do not graze on *Phaeocystis* (reviewed by Davidson and Marchant, 1992; Hansen and van Boekel, 1991; Hansen et al., 1994). *Phaeocystis* also appeared to have toxic or deleterious effects on some filter feeders (Estep et al., 1990; Schnack et al., 1985; Aaneson, et al., 1998; Pieters et al., 1980).

Analysis of food quality in Antarctic *Phaeocystis* has focused on lipids. Antarctic *Phaeocystis* strains are generally low in the essential fatty acids 22:6(n-3) and 20:5(n-3) (Nichols et al., 1991; Virtue et al., 1993). Single *Phaeocystis* contains relatively higher proportions of these fatty acids (Nichols et al., 1991; Pond et al., 1993; Virtue et al., 1993), but is poorly grazed by *E. superba* (Chapter 1), probably due to its small size ((McClatchie and Boyd, 1983; Boyd et al., 1984; Quetin and Ross, 1985; Ishii et al., 1985).

Few studies have addressed *Euphausia superba*'s nutritional requirements, and the importance of dietary lipids is unclear. Virtue et al. (1993) found that krill fed *Phaeocystis* sp. and *Phaeodactylum tricornutum* had similar percentages of the essential fatty acid 20:5 (n-3), even though concentrations of this fatty acid were much higher in *P. tricornutum*. They suggested that krill can convert shorter chain

fatty acids into 20:5 (n-3), reducing the importance of these "essential" fatty acids in their diet. During grazing experiments of *E. superba* on *Isochrysis* sp. and *Thalassiosira weissflogii*, Pond et al. (1995) reported considerable alteration of the ingested lipid pool, high turnover rate for lipids, and the use of ingested lipids primarily for energy production rather than storage. Possibly, protein content of food is more important for *E.superba* than lipids. For example, larval *E. superba* (primarily furcilia stage 6) exhibited high turnover of carbon and conservation of nitrogen (Frazer et al., 1997) suggesting that proteins are more limiting than lipids for *E. superba*, and C:N ratios may be a predictor of food quality. Additional studies which monitor assimilation, growth, condition and egg production of *E. superba* fed different Antarctic phytoplankton species are needed to test the link between selectivity and nutrition. Particular attention must be paid to both the krill's life stage and the season, since nutritional requirements may vary considerably depending upon whether a krill is using its food resources for growth, reproduction, or winter maintenance. Similarly, growth conditions of phytoplankton, such as light level, day length, temperature and nutrients, may significantly

affect its chemical composition (Hawes, 1990; Davies et al., 1992; Verity et al., 1988) and thus its nutritional value.

Even if diatoms are preferred, the complete lack of grazing on cryptophytes when they are virtually the only food available is puzzling. However, this behavior may reflect an adaptive strategy. According to optimal foraging theory, animals have evolved mechanisms to maximize their energy intake (MacArthur and Pianka, 1966) or some more relevant measure of food value (Rapport, 1981). Accordingly, mobile foragers may respond to cues indicative of "good food" by remaining in a patch and feeding, and to cues indicative of "poor food" by searching for an alternate food resource. In the nearshore region of the Antarctic Peninsula, cryptophytes were associated with near-surface, low saline water, especially glacier run-off (Moline and Prézelin, 1996), while diatoms were associated with more saline water (Moline et al., in prep) found deeper or further offshore. Perhaps krill minimize feeding on cryptophyte-dominated surface waters and swim downward or offshore, where they are likely to encounter higher proportions of diatoms. In contrast, when cryptophytes are in mixtures with diatoms, the krill stay and feed because desirable food (i.e. diatoms) is available, and cryptophytes are

ingested along with the preferred diatoms. Certainly, euphausiids are highly mobile and capable of locating patchy food resources (Price, 1989), although mechanisms for detection are not known.

Selectivity may be due to factors other than size and nutrition. In particular, chemical defenses of phytoplankton may play a role. For example, *Phaeocystis* contains acrylic acid (Sieburth, 1960) and dimethylsulphide, either of which could deter grazers (Estep et al., 1990).

Just as phytoplankton community composition influences krill grazing, selectivity by krill can impact phytoplankton community composition. Kopczynska (1992) suggested that krill grazing, along with physical mixing, shifted the phytoplankton assemblage towards flagellates. In this study, krill selected diatoms even when diatoms were rare, suggesting that krill may suppress diatom blooms, and that the influence of krill on diatom populations may be out of proportion to average grazing rates measured for a mixed phytoplankton community.

CONCLUSION

Antarctic krill, *Euphausia superba*, selectively grazed diatoms in preference to both prymnesiophytes and cryptophytes. The level of selectivity demonstrated cannot be attributed to particle size differences alone, and appears to involve more active mechanisms. The specific advantages of diatoms as a food resource are unknown and merit further study.

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Table 1. Solvent gradients for HPLC. See text for identification of Solvents A, B and C.

TIME	%A	%B	%C
0.0	100	0	0
0.1	100	0	0
2.0	0	100	0
2.6	0	75	25
13.6	0	30	70
18.0	0	30	70
20.0	0	100	0
22.5	100	0	0
30.0	100	0	0

Table 2. Initial conditions, clearance rates, and ratio changes for experiments. See Figs. 1, 3, 4, and 5 for further details of % change in ratios. Asterisk (*) indicates experiments with no acclimation period. Abbreviations: ALLO=Alloxanthin, HF=19'-hexanoyloxyfucoxanthin, FUCO=fucoxanthin, *T. a.=Thalassiosira antarctica*, *P. a.=Phaeocystis antarctica*, cryp=cryptophytes, *Pha=Phaeocystis*, diat=mixed diatoms.

EXPT	ALLO:HF:FUCO Initial conc	Phytoplankton	Initial conc chl a	Clearance rates				% change in ratio	% change in ratio
				chl a	FUCO	HF	ALLO	HF:FUCO	ALLO:FUCO
CULTURE MIXTURES									
3-07	(0.0): 0.5: 1.0	1:1 *P.a.: T.a.*	3.40	0.53	0.45	0	0	16%	
3-07	(0.0): 0.5: 1.0	1:1 *P.a.: T.a.*	3.40	1.20	1.45	0.55	0	75%	
3-10	(0.0): 0.3: 1.0	1:1 *P.a.: T.a.*	5.00	1.35	1.70	0.43	0	106%	
3-13	(0.0): 0.7: 1.0	1:1 *P.a.: T.a.*	6.60	0.23	0.76	0.39		11%	
3-06*	(0.0): 0.4: 1.0	1:1 *P.a.: T.a.*	8.41	1.87	1.68	0.74	0	369%	
3-16*	(0.0): 0.3: 1.0	1:1 *P.a.: T.a.*	18.40	0.45	**	**	0	23%	
NATURAL ASSEMBLAGES									
3-03 A	1.1: 1.4: 1.0	Cryp, *Pha.*, diat	2.65	0.53	0.72	0.45	0.27	65%	118%
3-03 B	1.2: 1.3: 1.0	Cryp, *Pha.*, diat	2.99	0.15	0.22	0.06	0.11	31%	22%
4-06 A	2.6: 0.6: 1.0	Cryp, *Pha.*, diat	3.02	0.05	0.08	0	0	9%	0%
4-06 B	2.3: 0.5: 1.0	Cryp, *Pha.*, diat	2.95	0.00	0.04	0	0	9%	13%
3-01 A	4.2: 1.8: 1.0	Cryp, *Pha.*, diat	6.70	0.00	0	0	0	0%	0%
3-01 B	7.9: 1.5: 1.0	Cryp, *Pha.*, diat	6.70	0.00	0	0	0	0%	0%
3-04 A	(0.0): 1.0: 1.0	*Pha.*, diat	0.73	0.03	0	0	0	0%	
3-04 B*	(0.0): 1.0: 1.0	*Pha.*, diat	0.74	0.07	0	0	0	0%	

Table 3. Pigment ratios for cultures used in experiments. See text for abbreviations.

Species	Description	FUCO: chl a	HF: chl a	HF: FUC
P. antarctica	mostly sm col (20-100 µm)	0	0.19	N/A
P. antarctica	mostly sm col (20-100 µm)	0	0.40	N/A
P. antarctica	mix, sm col and single	0.07	0.80	11.4
T. antarctica	25 µm diam cells, 1/3 in chains	0.69	N/A	N/A
T. antarctica	25 µm diam cells, 2/3 in chains	0.64	N/A	N/A

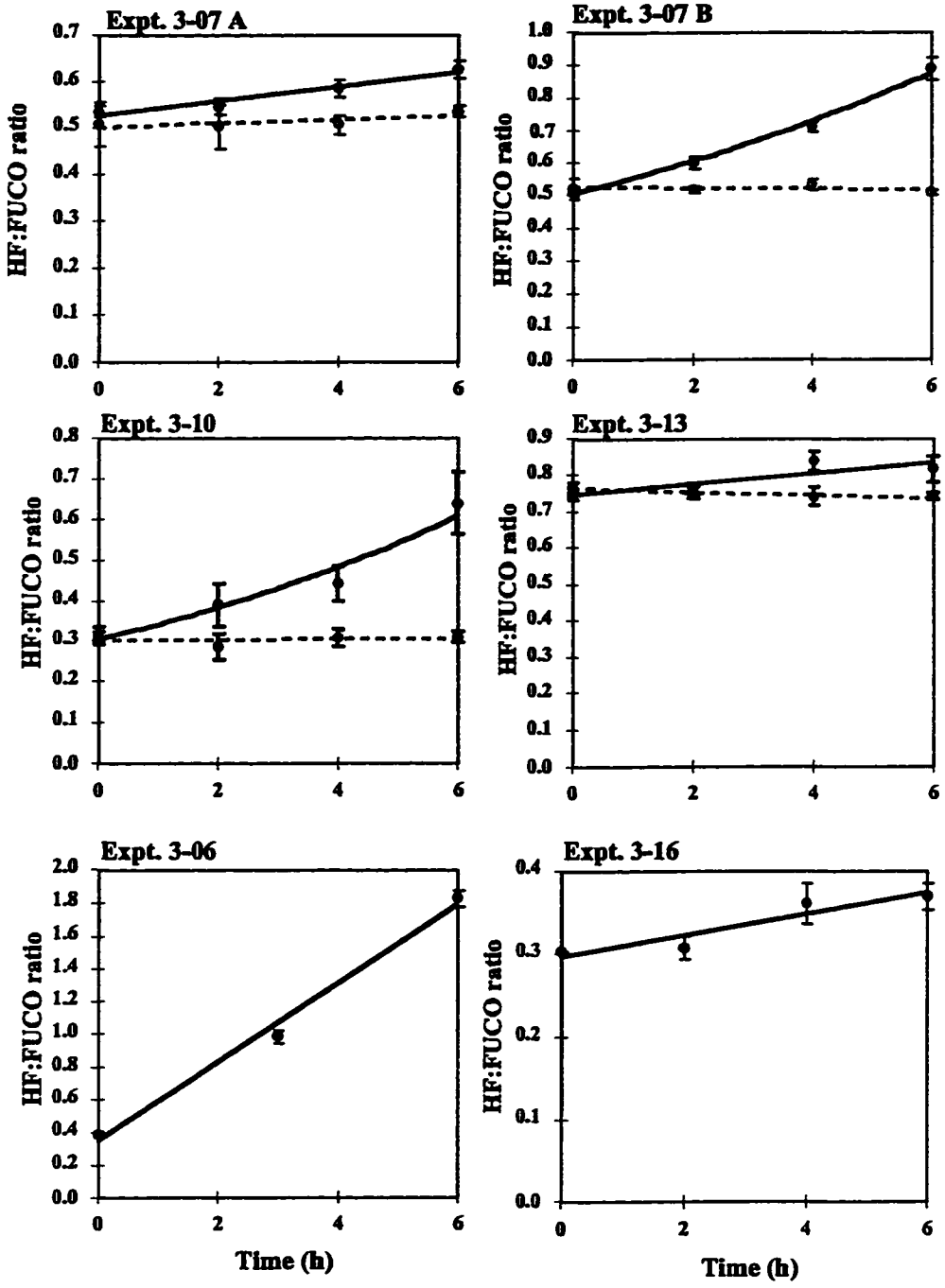

Expt. 3-07 A
Expt. 3-07 B
Expt. 3-10
Expt. 3-13
Expt. 3-06
Expt. 3-16
HF:FUCO ratio
Time (h)

Fig. 2. **Percent change in HF:FUCO ratio as a function of clearance rate (μg chl a/g wet wt.-h) for 1:1 mixtures of *Phaeocystis antarctica* and *Thalassiosira antarctica*.** Each square represents an individual experiment. The gray square is a high outlier. The equation is $y=6.76e^{2.08}$, $r^2=0.98$, outlier included. The equation is $y=7.24e^{1.97}$, $r^2=0.96$, outlier excluded.

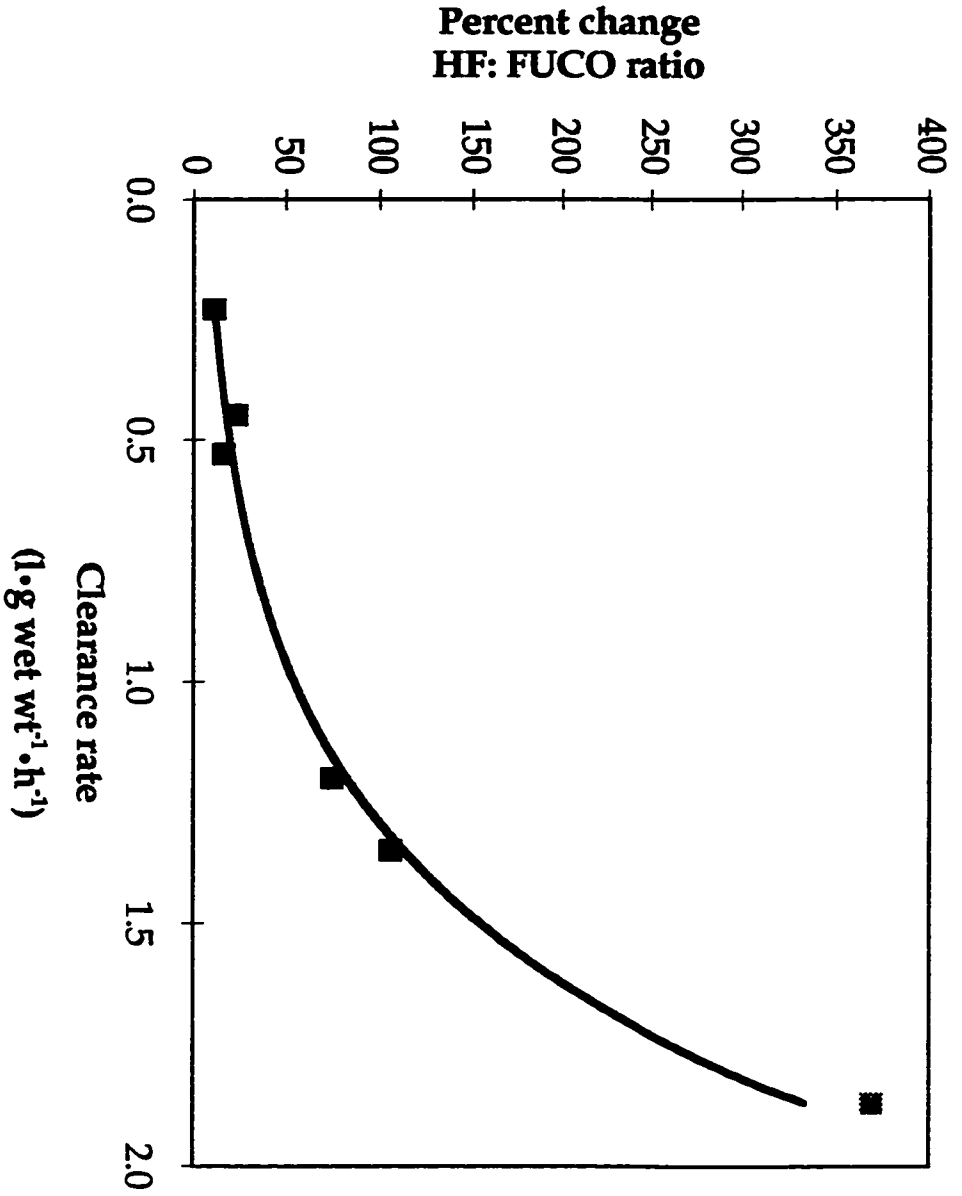
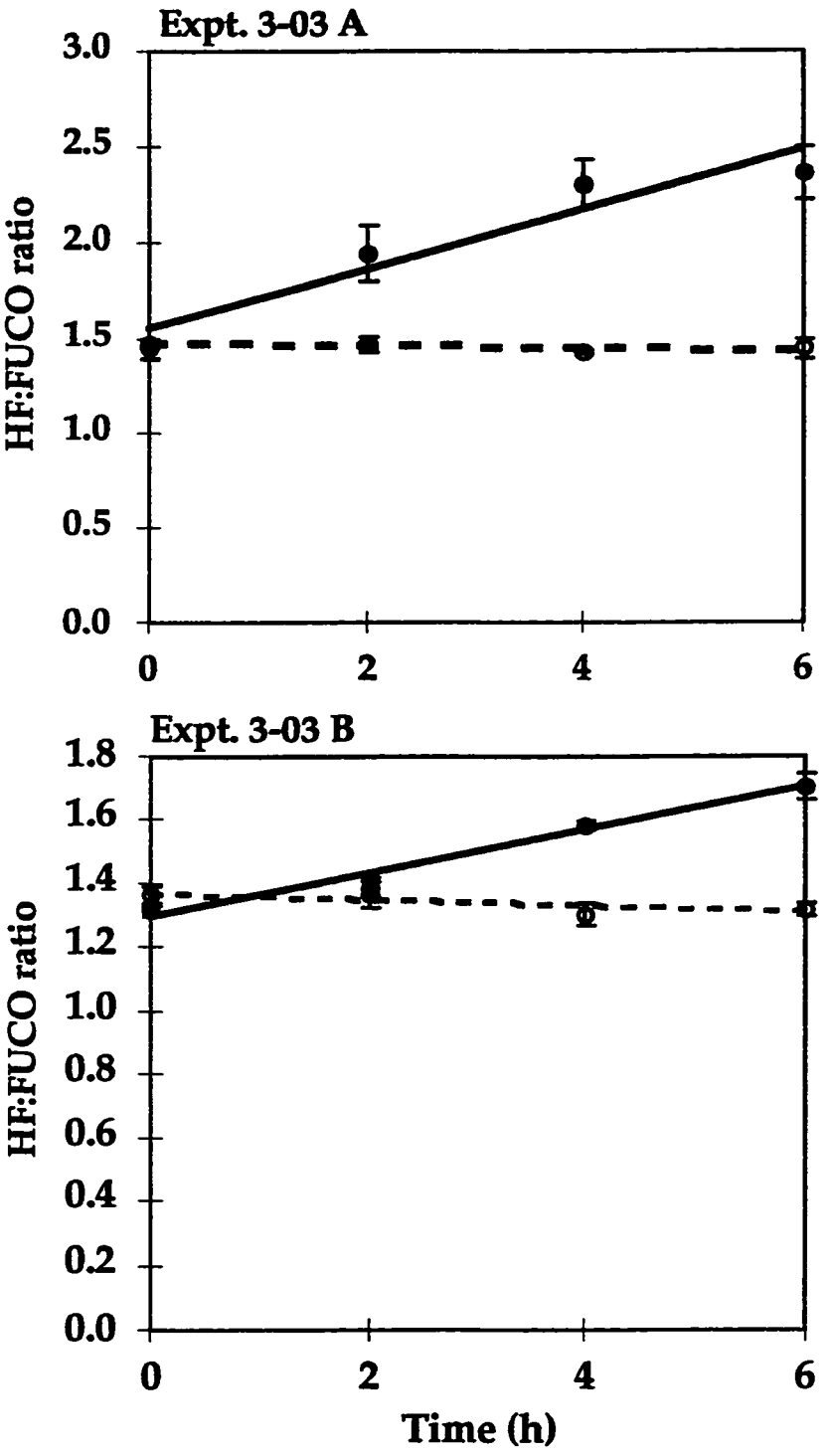

Percent change
HF: FUCO ratio
0
50
100
150
200
250
300
350
400
0.0
0.5
1.0
1.5
2.0
Clearance rate
(l•g wet wt-1•h-1)

Fig. 3. **HF:FUCO ratios for natural phytoplankton assemblages with approximately equal ALLO:HF:FUCO ratios.** Open circles and dashed lines show control periods (no krill), solid circles and lines show grazing periods (with krill). For both experiments, the slope for the grazing period was significantly different than the slope for control period (test for homogeneity of slopes, $p=0.000$). Slopes were not significant for either control period (regression analysis, $p>0.15$ for both).


Expt. 3-03 A
HF:FUCO ratio
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Expt. 3-03 B
HF:FUCO ratio
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
Time (h)

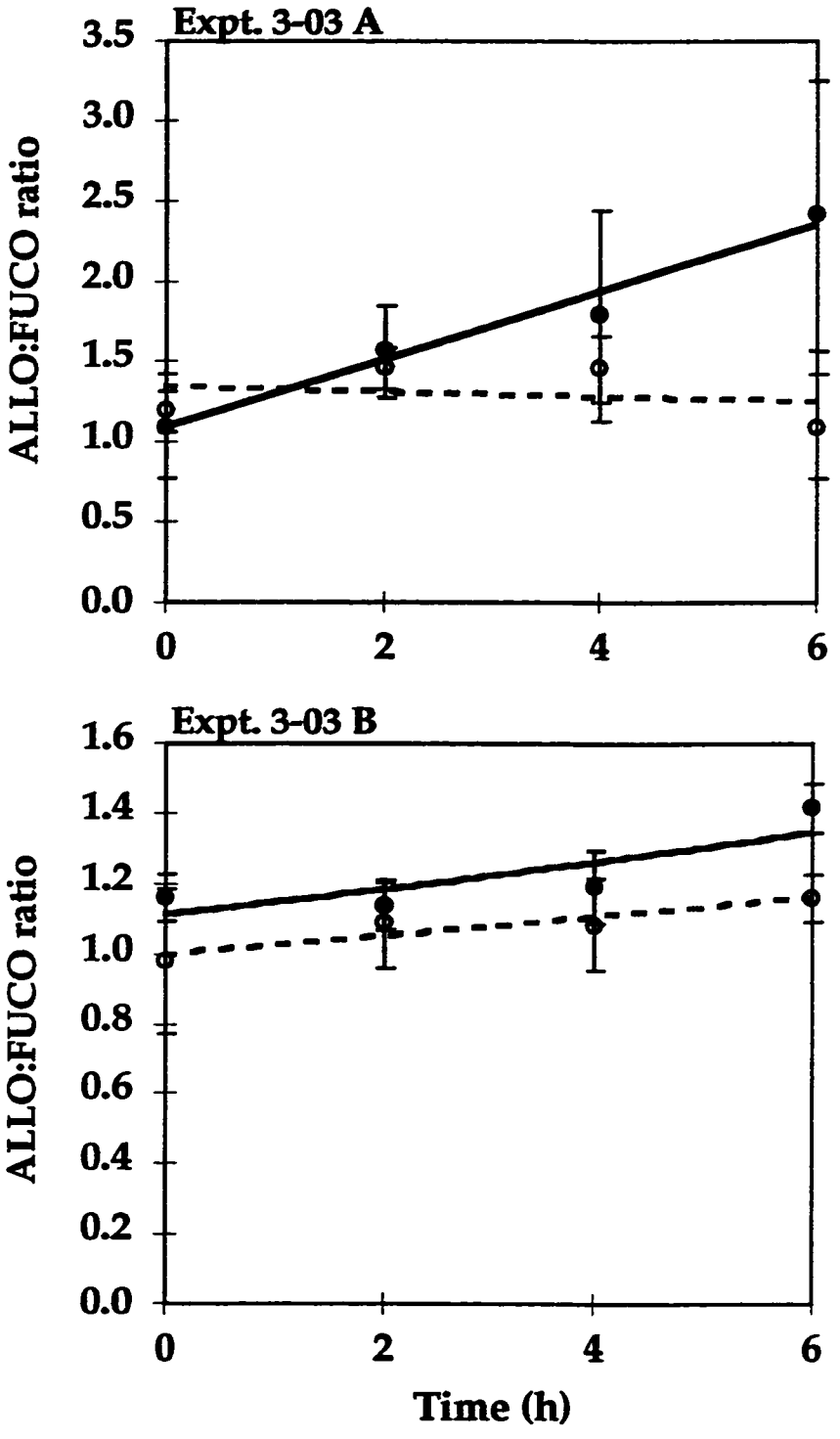
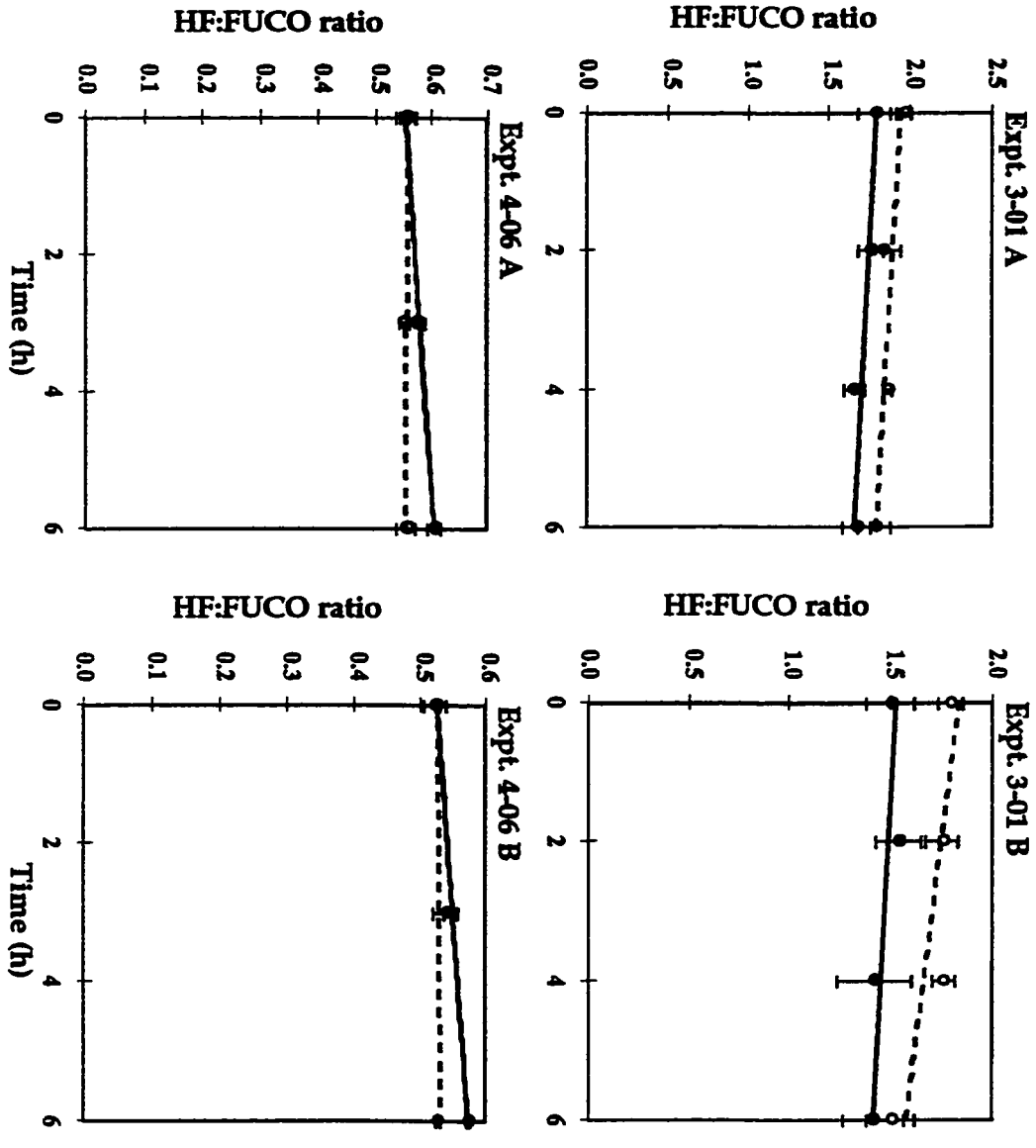

Expt. 3-03 A
ALLO:FUCO ratio
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Expt. 3-03 B
ALLO:FUCO ratio
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
Time (h)

Fig. 5. **HF:FUCO ratios for natural phytoplankton assemblages with relatively high cryptophyte concentrations.** Open circles and dashed lines show control periods (no krill), solid circles and lines show grazing periods (with krill). For Expt. 3-01 and 3-02, slopes are not significant for either the control or grazing periods. For experiments 4-06 A and 4-06 B, the slopes for the grazing periods were significantly different than the slopes for control periods (test for homogeneity of slopes, $p \leq 0.01$). The control periods did not have significant slopes ($p \geq 0.8$).


HF:FUCO ratio
Expt. 3-01 A
HF:FUCO ratio
Expt. 4-06 A
HF:FUCO ratio
Expt. 3-01 B
HF:FUCO ratio
Expt. 4-06 B
Time (h)
Time (h)

CHAPTER 4

An immunochemical assay for *Phaeocystis* in the stomachs of field-collected Antarctic krill (*Euphausia superba* Dana)

INTRODUCTION

The prymnesiophyte *Phaeocystis antarctica* is a potentially important food resource for the Antarctic krill, *Euphausia superba*, since it occurs in large blooms in the Antarctic (Fryxell et. al., 1988; Bidigare et. al., 1996) and its range of colony sizes overlaps the range effectively grazed by *E. superba* (Meyer and El-Sayed, 1983; Ishii et. al., 1985; Quetin and Ross, 1985). In laboratory experiments, krill cleared and ingested relatively small *P. antarctica* colonies (those between 50-100 μm diameter) at rates comparable to *Thalassiosira antarctica* (Chapter 1), while grazing single cells and larger colonies at lower rates. Krill also selected diatoms over *P. antarctica* within mixtures, even for the size of *P. antarctica* ingested at rates similar to those on *T. antarctica* (Chapter 3).

To extend the implications of these laboratory experiments, we need to determine the extent of *P. antarctica* consumption by *Euphausia superba* in the field. One approach has been the microscopic examination of *E. superba* stomach contents (Pavlov, 1969; Zhu, 1989; Opalinski et al., 1997). However, *E. superba* macerates its food prior to ingestion, and substantial digestion occurs within its stomach

One limitation of immunochemical gut analyses is that their previous use has been qualitative, i.e., presence or absence of the food item. However, some degree of quantification can be achieved by looking at the percent of animals which have a response to the prey antiserum (Theilacker, 1988; Theilacker et al.,1993) since assays are generally sensitive enough to detect responses even in very small animals. Also, there is potentially a quantitative relationship between prey protein and the immunochemical response, even for polyclonal antibodies directed against whole cells (Ohman et al., 1991). Even more promising, Hentschel and Feller (1990) obtained a quantitative immunochemical response of a polyclonal antibody to prey protein within white shrimp guts, and suggested that the antiserum response was not affected by digestion of the prey protein.

The purpose of this study was to develop a quantitative method to assess grazing by *Euphausia superba* on *Phaeocystis antarctica* in the field. To accomplish this, an enzyme-linked immunosorbent assay (ELISA) for *P. antarctica* was first developed and tested on *E. superba* with known feeding histories. Then, this assay was used to assess *P. antarctica* ingestion by krill collected on the shelf west of the Antarctic Peninsula in the austral summer (December 1996-February 1997).

METHODS

Overview

Several steps were required to develop a quantitative immunoassay for *P. antarctica* in krill stomach extracts. 1) Antiserum to *P. antarctica* was produced in rabbits. 2) The antiserum was screened with two qualitative assays to make sure it contained antibodies specific for *P. antarctica,* with minimum cross-reactivity to other types of phytoplankton. 3) One of the qualitative assays was further developed into a quantitative assay, and tested on phytoplankton extracts and krill with known feeding histories. 4) The quantitative assay was tested on mixtures of krill stomach extracts from krill fed only *P. antarctica* or only *Thalassiosira antarctica.* From these assays, a standard curve was generated which allowed for conversion from antiserum response values to percentages of stomach extract derived from krill fed*P. antarctica.* 5) Stomach extracts from field-collected krill were quantitatively tested for antiserum activity. The percentage of stomach extract attributable to grazing on *P. antarctica* was estimated for each station from the standard curve.

Production and collection of antisera

Preparation of *Phaeocystis antarctica* inoculum. Two strains of *P. antarctica*, one isolated from Palmer Station, Antarctica (by D. Karentz, referred to as Strain A) and the other isolated from McMurdo Sound (by M. Putt, Provasoli-Guillard Center for the Culture of Marine Phytoplankton, CCMP 1374, referred to as Strain B) were used to inoculate rabbits. Cultures were grown in f/2 medium minus silica (Guillard and Ryther, 1962) at 0.5-2.0 °C, and illuminated with cool white fluorescent lights on a variable light cycle which roughly corresponded to seasonal light conditions at 64° S latitude. Strain A was used for the initial inoculation and first booster, and Strain B was used for the second booster.

Cells and colonies of *Phaeocystis antarctica* were concentrated by centrifuging at 150 g for 4 minutes. The supernatant (primarily seawater) was siphoned off and discarded, and the collected material was centrifuged at 9000 g for two 2-3 min periods in order to separate the *P. antarctica* colony matrix from the embedded cells (Guillard and Hellebust, 1971) and thus minimize the amount of matrix in the

inocculum. The supernatant was removed until there was approximately a 50:50 mixture (by volume) of supernatant and cells. The sample was then shaken and mixed in a syringe with a wide-bore (16-gauge) needle to re-suspend the cells, then transferred to a cryovial and frozen in liquid nitrogen. Just prior to injection, the cell concentrate was thawed and a 1:1 mixture made with 50% (by volume) *P. antarctica* concentrate and 50% Freund's Incomplete Adjuvent (FIA; Sigma). The mixture was emulsified in a syringe with a wide bore needle.

Preparation of antisera. Two male New Zealand white rabbits were bled prior to inoculation to obtain unimmunized, control serum ("normal serum"). Then, they received multiple subcutaneous injections, totaling 1.0 ml, of the 1:1 *Phaeocystis antarctica*: FIA emulsion. Booster injections, identical to the initial inoculation in volume and delivery, were given at one month and again at approximately one year after the initial inoculation. Rabbits were bled for antiserum (1% rabbit body weight) 10-14 days after the first and second booster inoculations (Dresser, 1986) then exsanguinated to harvest additional antiserum 24 days after the second booster.

The blood was processed following a procedure modified from Hudson and Hay (1989). After collection, the blood was allowed to clot for 1 h at room temperature to separate serum from insoluble components. The initial clot was pulled away from the glass centrifuge tube with a sterile syringe and the blood was left to clot further at 4°C. Then, the serum was drawn out with a syringe and centrifuged at 150 g for 5 min, then at 350 g for 15 min to pellet any erythrocytes left in the serum. The supernatant (serum) was carefully siphoned off the erythrocytes, and sterilized by filtration through a 0.2 μm cellulose acetate sterile syringe filter (Nalgene) into a sterile, plastic centrifuge tube. The serum was then heated in a 56 °C isotemp oven for 30 min to deactivate the complement. A working portion of the serum was stored at 4 °C, while the rest was aliquoted (0.5-1.0 ml) into sterile centrifuge tubes and stored at -20 °C.

Qualitative screening for *Phaeocystis antarctica* antibodies

Pre and post-inoculation sera (referred to as "normal serum" and "antiserum" respectively) were initially screened for anti-*Phaeocystis antarctica* activity using the immunofluorescence technique of Campbell et al. (1983). This technique is a type of antibody capture assay (Harlow and Lane, 1988), and includes the four major steps common to all three immunoassays in this study (Fig. 1). The assay was carried out on filtered phytoplankton cultures, substituting Poretics 0.2 μm PCTE black filters for the iraglin black stained filters used by Campbell et al. After filtration, the filters were incubated with 1:10 dilutions of the primary antiserum or normal serum in phosphate buffered saline (PBS), followed by incubation with a 1:20 dilution of fluorescein isothiocyanate (FITC) labeled secondary antibody produced against rabbit IgG antibodies (Sigma). (See Campbell et al., 1983, for details of blocking steps, washes, and incubation times.) The filters were examined with epifluorescent microscopy (Zeiss Axiovert or Nikon epifluorescent scope, blue excitation filter), which allowed for qualitative determination of both the location and relative degree of primary antibody binding. These sera were tested against both strains of

P. antarctica as well as *Thalassiosira antarctica* (a diatom) to determine whether antiserum from either rabbit contained antibodies specific for *P. antarctica.*

After determining that antibodies to *P. antarctica* were present in the antisera, the antisera were further tested against whole cell concentrates from other phytoplankton species to determine the extent of cross-reactivity. Species evaluated included three prymnesiophytes: *Pavlova gyrans* (University of Texas, Strain UTEX LB992), *Isochrysis galbana* (UTEX LB987), and *Emiliana huxleyi* (UTEX LB1016) and two Antarctic diatom species: *Thalassiosira antarctica* and *Corethron criophilum,* both isolated by the author from waters near Palmer Station, Antarctica. *T. antarctica* and *C. criophilum* were several times more concentrated than the prymnesiophytes, because they concentrated more readily during centrifugation. Since this was a qualitative assay, no attempt was made to make to equalize these concentrations.

In order to test a large number of samples at once, a qualitative enzyme linked immunosorbent assay (ELISA) using 96-well polystyrene plates was developed (Harlow and Lane, 1988). Individual wells were first coated with 0.1% poly-L-lysine (Sigma) according to

manufacturer's instructions to promote attachment of cells. Concentrated phytoplankton (whole cells) were incubated in wells for 1 h at room temperature, with ten wells per phytoplankton type. After incubation, the wells were aspirated and rinsed with filtered seawater. The wells were then blocked for 1 h with 100 µl of 3% bovine serum albumin (BSA) in sterile Tris-buffered saline (TBS; 25 mM, pH=8.0), after which the blocking solution was aspirated. Each phytoplankton type was then incubated for 1 h with duplicates of the primary antiserum diluted 1:10, 1:100 and 1:1000 in TBS, and duplicates of the normal serum diluted at 1:10 and 1:100 in PBS. The incubation was followed by four 10-min TBS rinses. The wells were then blocked as previously described, then incubated for 1 h with the secondary antibody, goat anti-rabbit conjugated with alkaline phosphatase, 1:10,000 in TBS. This incubation was followed by four 10 min TBS rinses. The wells were incubated for 30 min with BCIP/NBT, an alkaline phosphatase substrate with a purple precipitate as a reaction product. This reaction was stopped with 2 mM EDTA in TBS. Reaction product color was evaluated qualitatively by eye as 3=dark, 2=medium, 1=light and 0=none.

Tests of antibody activity for extracts of phytoplankton and krill stomachs

After initially screening the antiserum for anti-*Phaeocystis antarctica* activity and cross-reactivity, the microplate method was further developed into a quantitative ELISA for measuring anti-*P. antarctica* activity in phytoplankton and krill stomach extracts.

Preparation of extracts. Concentrated phytoplankton cultures, stored at -80 °C, were thawed and macerated with a miniature tissue grinder (Fisher Scientific) and centrifuged at 1000 g. The supernatant was used for protein and antibody tests. The two *Phaeocystis antarctica* strains, two diatom species (*Thalassiosira antarctica* and *Corethron criophylum*) and one non-*Phaeocystis* prymnesiophyte (*Pavlova gyrans*) were included in the assays.

Krill with known feeding histories, obtained from grazing experiments on *Phaeocystis antarctica* and *Thalassiosira antarctica* (Chapter 1) were frozen at -80°C for use as standards in the antibody assays. The stomachs were dissected from the krill, and each extract

was prepared by homogenizing stomachs from 10-25 krill (depending upon krill size) in approximately 100 µl of DI water. The stomach extract was frozen until assayed.

Protein determination of extracts. Phytoplankton and krill extracts were assayed for total protein, in order to match the total protein for all samples in immunoassays. The Lowry et al. (1951) method was modified for microplates by reducing the reagent volumes. Reagent proportions did not differ from the original method. Proteins were dissolved in NaOH prior to the addition of alkaline copper solution (Lowry et al., 1951). Dilutions of bovine serum albumin (BSA) were used as protein standards, and a log/log plot was used to obtain the most accurate standard curve (Peterson, 1977). For each phytoplankton or krill stomach extract, two samples at each of three dilutions were assayed, and protein values calculated from the standard curve. Extracts were then diluted to final protein concentrations of 6.5 µg/30 µl of 10 mM Tris-HCl/1 mM EDTA extraction buffer (Theilacker et al., 1986), which were used in ELISA assays.

Quantitative ELISA for *Phaeocystis antarctica* antigens. The quantitative ELISA was based on guidelines given by Harlow and Lane (1988). Microplates were prepared by lining individual wells with nitrocellulose paper, which had been cut with a sterile hole-punch. Nitrocellulose paper was chosen for its proven ability to bind euphausiid stomach extracts for immunochemistry (Theilacker et al., 1986). Thirty microliter aliquots of phytoplankton or krill stomach extract, adjusted for protein content as described previously, were added to individual wells, with ten replicates per plate. The extract was incubated in the wells for 30 min at room temperature, and then dried at 54 °C for 30 min, after which the plate was placed in a plastic bag with desiccant and refrigerated overnight (4 °C) to promote further evaporation. In the morning, excess fluid, approximately 5 µl/well, was aspirated from each well, and the plate was returned to the drying oven for 10 min.

To rinse excess material not attached to the nitrocellulose paper, 3% BSA in DI water was added to each well (100 µl/well) and allowed to stand for 15 min. One hundred µl of TBS was added to each well for an additional 15 min, after which the wells were aspirated. Wells were then blocked with 3% BSA in TBS (100 µl/well) for 1 h followed by

aspiration. Next, wells were incubated with primary antiserum for 1 h. For each type of phytoplankton or krill extract, 5 wells were incubated with 50 µl of a 1:500 antiserum dilution (in 3% BSA/TBS) and the other 5 wells were incubated with 50 µl of 1:500 normal serum as a control for non-specific binding. (The 1:500 concentration was determined empirically in preliminary tests to maximize the difference between normal serum and antiserum binding.) After incubation, wells were rinsed in TBS 3 times, 10 min per rinse. Wells were again blocked with 3% BSA in TBS as previously described, then incubated for 1 h with 50 µl of 1:10,000 dilution (in 3% BSA/TBS) of the secondary antibody, goat anti-rabbit IgG with alkaline phosphatase conjugate (Sigma). After this incubation, wells were rinsed in TBS 3 times, 10 min per rinse.

To quantify the assay, each well was incubated for 60 min in the dark with 200 µl of TBS buffered p-nitrophenyl phosphate (pNPP) liquid substrate (Sigma). This reaction was stopped by adding 50 µl of 3M NaOH to the wells and agitating the plate on a shaker table for 1 minute. 180 µl from each well was moved to a clean polystyrene microplate (Corning) and absorption values (OD) at 405 nm measured with a Molecular Devices UV Max or Spectra Max microplate reader.

The net antiserum response equaled the mean OD of wells with antiserum minus the mean OD of wells with normal serum. Standard errors for the net response were also calculated, from the standard errors of the antiserum and normal serum responses (Freedman et al., 1991).

Standard curve for krill stomach contents. One goal of the ELISA assay was to estimate the percentage of *Phaeocystis antarctica* contributing to the overall stomach content of a krill. Accordingly, a standard curve was constructed from stomach extracts of krill which had been fed either *P. antarctica* or *Thalassiosira antarctica* in laboratory grazing experiments. These extracts were first made equal for protein concentration by dilution in the extraction buffer, and then mixed together so that there were six different concentrations of *P. antarctica* per total extract: 0%, 20%, 40%, 60%, 80% and 100% *P. antarctica* extract. The ELISA was conducted with each concentration treated as a separate extract. The resulting values were fitted to a logarithmic function.

Assay for *Phaeocystis antarctica* in the stomachs of field-collected krill

The quantitative ELISA was used to assess the extent of grazing on *P. antarctica* by Antarctic krill. Krill were collected with a 2-m Metro net (mesh size approximately 700 μm) for immunological analysis at 22 stations within the Palmer Long Term Ecological Research (LTER) grid west of the Antarctic Peninsula (Waters and Smith, 1992) during the January-February 1997 LTER cruise on the M/V Polar Duke and at two stations in the Palmer nearshore region during December, 1996 (Fig. 2). After collection, krill were individually frozen at -80 °C until used for the immunochemical assay. Stomach contents were extracted and assayed as described previously. Percent of stomach extract derived from *Phaeocystis* was estimated from the standard curve for stomach extracts of krill with known feeding histories.

RESULTS

Qualitative screening of antiserum against concentrated phytoplankton. Preliminary immunofluorescent screening of the antiserum induced to *Phaeocystis antarctica* showed a strong fluorescent response for *P. antarctica* primarily associated with the cell surface (not the colony matrix), and a much weaker response for *Thalassiosira antarctica.* There was a relatively low fluorescent response for the normal serum. Further testing of the antiserum using the qualitative ELISA showed strong reactivity with *P. antarctica,* minimal cross-reactivity with the diatoms *T. antarctica* and *Corethron criophilum* and no detectable cross-reactivity with three non-*Phaeocystis* prymnesiophytes: *Pavlova gyrans, Isochrysis galbana,* and *Emiliana huxleyi* (Table 1). Note that no attempt was made to equalize concentrations among phytoplankton types in this assay, and subsequent protein analysis showed that the diatoms were several times more concentrated than the prymnesiophytes (Haberman, unpublished data).

(Fig. 4a; $p<0.001$). In contrast, the response for the stomach extract from *Thalassiosira antarctica*-fed *E. superba* was only 1.3 times greater than its response to normal serum ($p<0.01$). The net antiserum responses for both experiments with *P. antarctica*-fed krill were approximately an order of magnitude greater than the net response for *T. antarctica*-fed krill (Fig. 4b).

When the stomach extracts from *Phaeocystis antarctica*-fed and *Thalassiosira antarctica*-fed krill were mixed at different percentages, the net serum OD values were a logarithmic function of the percent *P. antarctica*-fed krill extract in the mixture (Fig. 5) This function was used as a standard curve to evaluate the quantitative ELISA results for field-collected krill. Zero percent *P. antarctica* was considered as a one percent response for the curve fit. Stomach extracts with net OD values less than, or not significantly different from, this 1% response level (OD=0.121; t-test, $p>0.05$) were considered to be negative for *P. antarctica*.

Quantitative ELISA for field-collected *Euphausia superba*. *E. superba* stomach extracts from 4 of 22 stations tested from the January-February 1997 LTER cruise had positive responses to the antiserum

(Fig. 2), and estimates for percent *P. antarctica* in stomach extracts ranged from 36% to 100% (Table 2). Samples from these stations were collected within a four day time period, and all but one were collected in nearshore regions (Table 2). In addition, 1 of the 2 stations sampled from the Palmer LTER nearshore grid (December, 1996) had a positive, but low (<10%) response to the antiserum (Fig. 2, Table 2). These results indicated that *E. superba* grazed *P. antarctica* at approximately 20% of the stations surveyed.

DISCUSSION

The quantitative ELISA proved to be a sensitive assay for *Phaeocystis antarctica* in krill stomach extracts, with strong positive responses when only small percentages of extract was derived from *P. antarctica*-fed krill. In addition, the method apparently did not give false positive responses. For example, stomach extracts from krill fed *Thalassiosira antarctica* had a minimal response compared to the stomach extracts from krill fed *P. antarctica*. Also, there was a clear dichotomy between the positive and negative responses for field-collected samples, without intermediate values which would suggest

While cross-reactivity could be a source of false-positive results, molecular breakdown and a resultant loss of detectability could lead to false-negative results. This is of particular concern for krill compared to other zooplankton. For example, Antarctic krill are known to break down the plant pigment chlorophyll a into non-fluorescing end products at much higher rates than copepods (Perissinotto and Pakhomov, 1996), and in general, a higher degree of pigment breakdown occurs for larger zooplankters (Nelson, 1989). Similarly, loss of antiserum response may occur as antigen molecules break down within the krill's stomach, especially since some antibodies require a degree of tertiary molecular structure for epitope recognition (Harlow and Lane, 1988). On the other hand, Hentschel and Feller (1990) found that changes in antiserum response to oyster protein in the white shrimp proventriculum (stomach) were due to actual changes in oyster protein concentration (presumably due to absorption or movement out of the proventriculum) rather than changes in the antigenic response due to breakdown. Also, it is likely that the anti-*Phaeocystis antarctica* antibodies were produced against thermally degraded molecules in the first place, since the ambient temperature of *P. antarctica* is 0-4 °C, considerably lower than a rabbit's body temperature. In sum,

molecular degradation may not be a significant problem for krill stomach immunoassays, but controlled laboratory studies specific to krill and phytoplankton food sources are required to address this issue. Throughput and absorption rates of prey antigen should also be examined because they could similarly affect interpretation of results (Hentschel and Feller, 1990.)

While the immunoassay developed in this study was designed to detect *Phaeocystis antarctica* in krill stomachs, the quantitative ELISA could be readily adapted for other krill food resources. Since antibodies can be produced against plant, protist and animal material, this method could be useful for quantifying the relative levels of herbivory and omnivory in *E. superba*, a topic which has generated much recent interest (Atkinson and Snÿder, 1997; Pakhomov et al., 1997), and which is important for clarifying the Antarctic krill's trophic position and winter survival strategies.

CONCLUSION

The quantitative ELISA detected ingestion of *Phaeocystis antarctica* by the Antarctic krill, *Euphausia superba*, at approximately 20% of stations tested. These results suggest that *P. antarctica* was a relatively limited food resource for krill, both spatially and temporally, during December 1996-February 1997 in waters off the Antarctic Peninsula. Further development of the method is necessary to more accurately quantify the level of *P. antarctica* ingestion at locations where it was eaten by krill. In addition, to determine whether *P. antarctica* ingestion by *E. superba* was limited by scarcity of *P. antarctica* in the study region, or by selective feeding on other phytoplankton, these results must be compared to the distribution, concentration, and proportion of *P. antarctica* in the water column for these same times and locations. Finally, the ELISA technique holds promise for identifying and quantifying the roles of different food resources in the diets of krill and other zooplankton.

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Table 1. Qualitative ELISA, visual ranking of reaction product for concentrated phytoplankton cells. 3=dark, 2=medium, 1=light, 0=none. AS=antiserum, Rabbit #420; NS=normal serum, Rabbit #420. Below the serum type are dilutions in Tris-buffered saline (TBS). N/A indicates no sample was run.

PHYTOPLANKTON SPECIES	SERUM TYPE AND DILUTION									
	AS 1:10		AS 1:100		AS 1:1000		NS 1:10		NS 1:100	
Phaeocystis antarctica B	3	3	3	3	2	2	0	0	0	0
Pavlova gyrans	0	0	0	0	0	0	0	0	0	0
Emiliana huxleyi	0	0	0	0	0	0	0	0	0	0
Isochrysis galbana	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A
Thalassioisira antarctica	1	1	0	0	0	0	0	0	0	0
Corethron criophylum	1	1	0	0	0	0	0	0	0	0

Table 2. Quantitative ELISA for field-collected stomach extracts from *Euphausia superba*. Station locations correspond to Fig. 2: the first three digits denote the transect line (200-600), and the second three digits denote location from nearshore to offshore at 20 K intervals. (For example, 200.000 is on the 200 line closest to the peninsula, southeast end of line). Special stations: MB= Marguerite Bay. BAS.1= between Adelaide I. and the Antarctic Peninsula. PAL= Palmer nearshore stations. Abbreviations: AS=antiserum, NS=normal serum. Percent *Phaeo* is the estimate of the percent of stomach extract derived from *Phaeocystis*, extrapolated from the standard curve (Fig. 5). **BOLD** indicates positive antiserum responses. Dashes indicate values which were not significantly different from controls.

STATION ID		AS RESPONSE (OD)		NS RESPONSE (OD)		NET ANTISERUM RESPONSE (OD)		PERCENT *PHAEO*
Date	Grid location	Mean	SE	Mean	SE	Mean	SE	
2/6/97	**MB**	**1.22**	**0.03**	**0.31**	**0.02**	**0.91**	**0.03**	**48%**
2/5/97	**BAS.1**	**1.20**	**0.02**	**0.30**	**0.02**	**0.90**	**0.03**	**45%**
2/3/97	200.000	0.46	0.02	0.31	0.04	0.14	0.05	-
2/4/97	**200.060**	**1.50**	**0.03**	**0.32**	**0.02**	**1.18**	**0.04**	**100%**
2/2/97	200.080	0.40	0.01	0.32	0.01	0.08	0.02	-
2/2/97	200.120	0.36	0.01	0.31	0.02	0.05	0.02	-
1/30/97	300.040	0.41	0.01	0.32	0.02	0.09	0.03	-
1/30/97	300.100	0.38	0.01	0.30	0.01	0.08	0.02	-
2/7/97	**400.000**	**1.21**	**0.03**	**0.35**	**0.03**	**0.85**	**0.04**	**36%**
1/27/97	400.040	0.42	0.02	0.31	0.03	0.11	0.03	-
1/27/97	400.100	0.36	0.02	0.34	0.01	0.02	0.03	-
1/28/97	400.140	0.40	0.03	0.37	0.02	0.03	0.03	-
2/8/97	440.000	0.49	0.02	0.33	0.01	0.16	0.02	-
1/12/97	500.060	0.36	0.01	0.29	0.01	0.08	0.01	-
1/13/97	500.160	0.39	0.01	0.31	0.00	0.08	0.01	-
1/13/97	500.180	0.35	0.01	0.31	0.01	0.04	0.01	-
1/14/97	500.200	0.34	0.01	0.31	0.02	0.04	0.03	-
2/11/97	550.030	0.34	0.01	0.33	0.01	0.01	0.02	-
2/12/97	596.014	0.41	0.01	0.34	0.03	0.06	0.03	-
1/16/97	600.040	0.37	0.01	0.37	0.03	0.00	0.03	-
1/16/97	600.100	0.32	0.01	0.35	0.02	-0.03	0.02	-
1/17/97	600.120	0.39	0.02	0.36	0.02	0.03	0.03	-
12/4/96	**PAL**	**0.82**	**0.01**	**0.32**	**0.01**	**0.50**	**0.01**	**6%**
12/16/96	PAL	0.41	0.01	0.33	0.02	0.08	0.02	-

Fig. 1. General protocol for an antibody capture assay.

Fig. 2. LTER sampling grid west of the Antarctic Peninsula, modified from Waters and Smith (1992). Numbered transect lines are 100 km apart, and stations along transect lines are 20 km apart. AN=Anvers Island, AD=Adelaide Island, MB=Marguerite Bay. Diagram indicates stations where krill were collected for immunochemistry during the January-February 1997 LTER cruise (triangles), and in the nearshore region of Palmer during December 1996 (squares). Stations where krill tested positive for grazing on *Phaeocystis* with the quantitative ELISA are indicated by the larger, filled triangles and squares.

60°S
62°S
64°S
66°S
68°S
70°S
80°W
75°W
70°W
65°W
60°W
55°W
200
300
400
500
600
MB

Fig. 3. Quantitative ELISA for phytoplankton extracts. Phaeo A: *Phaeocystis antarctica*, strain A; Phaeo B: *P. antarctica*, strain B; Prymn (Pav): *Pavlova gyrans;* Diatom (Thal): *Thalassiosira antarctica;* Diatom (Cor): *Corethron criophylum*. Serum responses are given in units of optical density (OD) at 405 nm. Error bars are 95% confidence intervals.

a. Comparison of antiserum (black bar and normal serum (grey bar) responses.

b. Net antiserum responses.

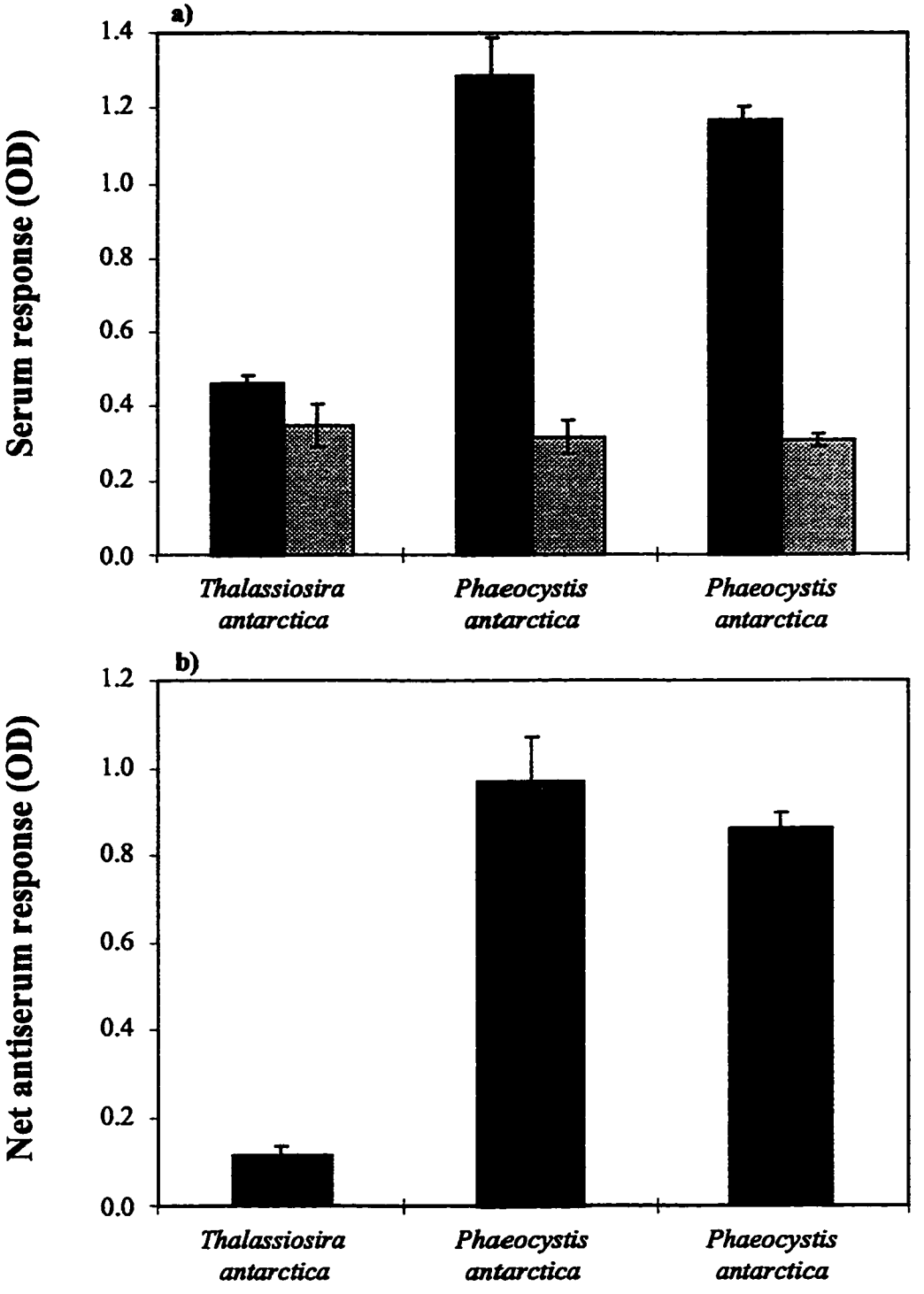
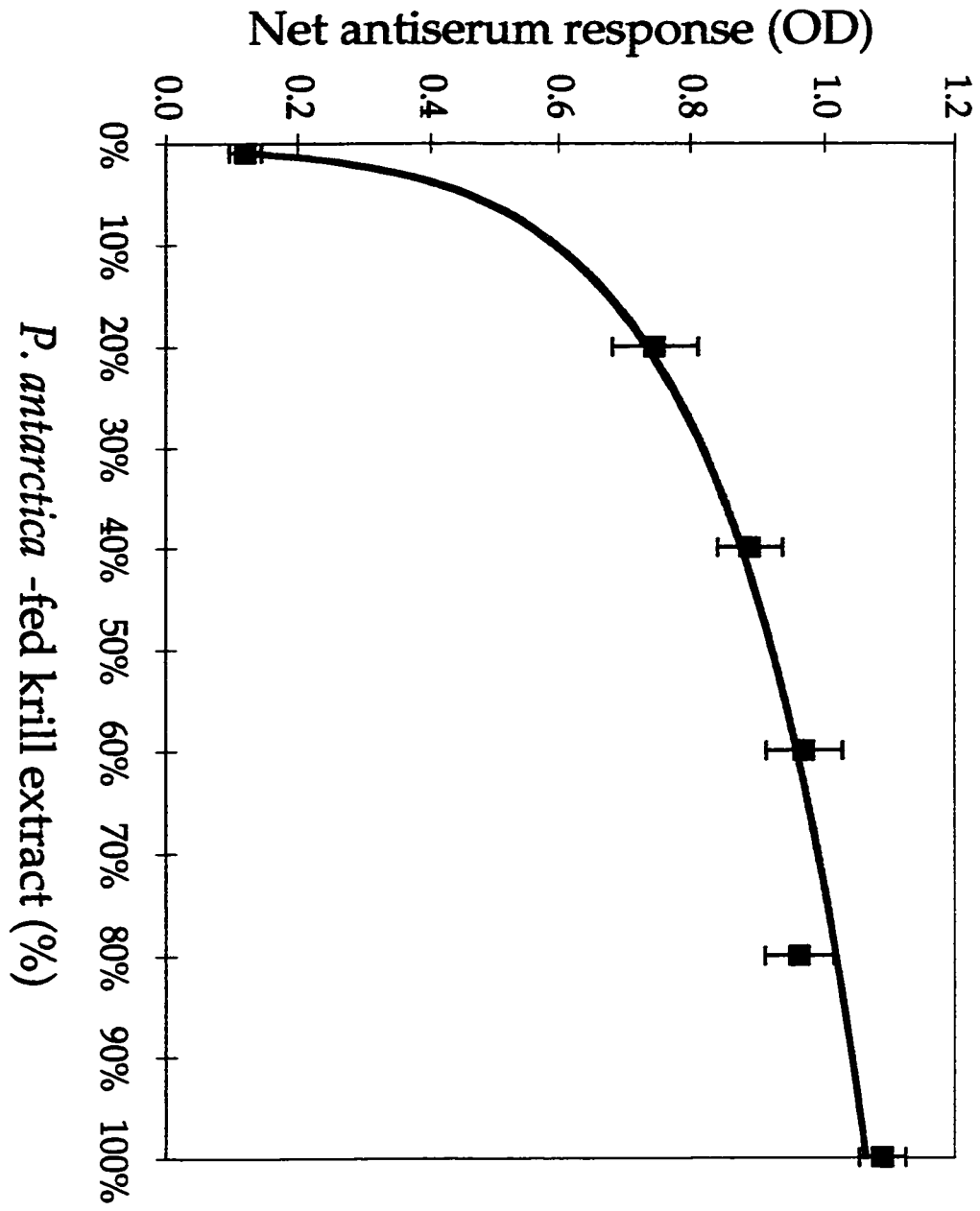

a)
Serum response (OD)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Thalassiosira antarctica
Phaeocystis antarctica
Phaeocystis antarctica
b)
Net antiserum response (OD)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Thalassiosira antarctica
Phaeocystis antarctica
Phaeocystis antarctica
Krill stomach extracts

Fig. 5. Stomach extract standard curve. Quantitative ELISA of stomach extract mixtures, with differing proportions of *Phaeocystis antarctica*-fed and *Thalassiosira antarctica*-fed krill extracts, expressed as the percent *Phaeocystis antarctica*-fed extract in the mixture. Extracts were equalized for protein concentration prior to mixing. Net antiserum response is in units of optical density (OD) at 405 nm. Error bars are 95% confidence intervals. Curve fit is logarithmic: $y=0.20 \ln(x)+1.06$, $r^2=0.99$.


Net antiserum response (OD)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
P. antarctica -fed krill extract (%)