ELSEVIER

Journal of Experimental Marine Biology and Ecology
283 (2003) 79–95

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

www.elsevier.com/locate/jembe

Diet of the Antarctic krill (*Euphausia superba* Dana): I. Comparisons of grazing on *Phaeocystis antarctica* (Karsten) and *Thalassiosira antarctica* (Comber)

Karen L. Haberman[a,*], Langdon B. Quetin[b], Robin M. Ross[b]

[a] *Biology Department, Western Oregon University, Monmouth, OR 97361, USA*
[b] *Marine Science Institute, University of California, Santa Barbara, CA 94306, USA*

Received 7 January 2002; received in revised form 18 September 2002; accepted 3 October 2002

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 *Euphausia 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 (100–500 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.

Keywords: Antarctic krill; Diet; *Euphausia superba*; Grazing; *Phaeocystis antarctica*; Phytoplankton

1. 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.,

* Corresponding author. Tel.: +1-503-838-8478; fax: +1-503-838-8072.
E-mail address: habermk@wou.edu (K.L. Haberman).

0022-0981/03/$ - see front matter © 2003 Published by Elsevier Science B.V.
PII: S0022-0981(02)00466-5

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, a major component of these blooms, are 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 and often dominate spring ice edge blooms along the Antarctic Peninsula (Fryxell and Kendrick, 1988; Bidigare et al., 1996), where *E. superba* is abundant in shelf waters (Ross et al., 1996). *Phaeocystis* can also be a major component of near-shore blooms in the Palmer Station region (Moline and Prézelin, 1996). Krill are frequently found in this coastal habitat (Lascara et al., 1999), which may be an important feeding ground for immature krill. Thus, *Phaeocystis* is a potentially important food resource for *E. superba*.

Qualitative evidence of grazing on *Phaeocystis* comes from three studies. Both Sieburth (1960) and Haberman et al. (2002b) detected *Phaeocystis* in the guts of krill. Also, Virtue et al. (1993) showed that krill can graze and assimilate *Phaeocystis* in the laboratory. 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 *Phaeocystis*. 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 rather than ingest 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 copepods (reviewed in Davidson and Marchant, 1992; Weisse et al., 1994). While results were highly variable and method-dependent, some conclusions of possible relevance to *E. 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 et al., 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 and 8 μm in diameter. As the bloom progresses, the proportion of the colonial form and the size of the colonies increases. Colonies are composed of nonflagellated cells imbedded in a high carbohydrate "gelatinous" matrix (Verity et al., 1988). 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, three species of *Calanus* were unable to graze actively growing *Phaeocystis* colonies, but consumed senescent ones (Estep et al., 1990). Since *E. superba* is known to more efficiently consume larger phytoplankton (Meyer and El-Sayed, 1983; Quetin and Ross, 1985; Ishii et al., 1985), its ability to graze *Phaeocystis* may also depend upon *Phaeocystis*' size and physiological state.

Given the prominence of *Phaeocystis antarctica* in regions of high krill biomass and its variable consumption by other zooplankters, it is important to quantify the role of *P. antarctica* as a food resource for krill. Additionally, since the relative proportions of *P. antarctica* and diatoms in these regions vary among seasons and years (Moline and Prézelin, 1996), studies of the krill's ability to graze *P. antarctica* may help explain interannual variability in krill growth and reproduction (Quetin et al., 1996; Ross et al., 2000).

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

2. Methods

2.1. Collection and maintenance of krill

Experiments were conducted during the austral spring and summer of 1993–1994, 1994–1995, and 1996–1997 at Palmer station, Antarctica, west of the Antarctic Peninsula. Krill were collected from a rubber skiff with a 1-m ring net and from the M/V Polar Duke with a 2-m square-framed net. Mesh size was 500 or 750 μm for nets and 500 μm for cod ends. Krill were maintained in 2.5-m-diameter flow-through seawater tanks at Palmer Station. Ambient water temperature ranged from −0.5 to 1.5 °C. Feeding conditions depended upon the phytoplankton in the incoming seawater, and ranged from 0.5 to 5.0 μg chl $a \cdot l^{-1}$. Highest mortality rates occurred in the first few days after collection, presumably due to damage during trawling. Accordingly, krill were held in these tanks for a minimum of 1 week to ensure that experimental krill were healthy. Experimental animals were chosen from the holding tank on the basis of size (approximately 35–40 mm) and healthy appearance, i.e. active swimming and/or feeding, transparent abdomen without white patches, and not missing eyes.

2.2. Maintenance and assessment of phytoplankton cultures

P. antarctica and *T. antarctica* cultures, both initially isolated from the Palmer Station region, were grown in 20-l clearboy containers at 1.5–2.0 °C illuminated by cool white fluorescent lights on a cycle of 18:6-h light/dark. Cultures were aerated using Silent Giant pumps, with air filtered through Gelman bacterial airvents to prevent contamination.

T. antarctica was grown in f/2 media (Guillard and Ryther, 1962). *P. antarctica* was grown primarily in f/2 media. However, specific media components were altered for some cultures in the attempt to grow large, colonial *P. antarctica*. Changes were made to: (1) media strength (Sieburth, 1960), (2) relative manganese levels (Davidson and Marchant, 1987), and (3) relative phosphate concentrations (Veldhuis and Admiraal, 1987). These variations did not have any apparent effects on colony formation and size. In another effort to produce larger *P. antarctica*, air flow into the clearboys was reduced, minimizing the degree of agitation inside the containers (Andrew Davidson, personal communication). The clearboys were not aerated for the initial 24 h after culture inoculation, after which

aeration levels were controlled by a bleed valve so that air entered at the rate of approximately 1 bubble/s. This method produced medium-sized colonies with dimensions primarily between 100 and 500 μm (Table 1). Larger colonies had both a spherical and an elongate component and resembled volumetric flasks. This low-level aeration technique

Table 1
Initial particle sizes of phytoplankton, and cell size and degree of chain formation for *T. antarctica* (T.a.) [experiments are grouped by *P. antarctica* (P.a.) type]

	Minimum particle dimension (median value, μm)		Maximum particle dimension (median value, μm)		Cell size and % chains, T.a. only	
	T.a.	P.a.	T.a.	P.a.	Cell size	% Chains
P.a. as single cells						
S-1	15	7	20	7	S	0
S-2	23	7	52	7	L	5
S-3	16	7	21	7	S	5
S-4	18	7	20	7	S	8
S-5	17	7	20	7	S	4
S-6	16	7	21	7	S	17
P.a. as small colonies						
SC-1	25	58	30	67	S	50
SC-2	24	86	45	106	S	56
SC-3	17	48	25	58	S	32
SC-4	25	48	46	58	S	71
SC-5	25	67	43	77	S	83
P.a. as medium colonies						
MC-1	17	106	31	125	S	26
MC-2	19	96	29	173	S	2
MC-3	18	144	29	192	S	5
MC-4	18	154	29	259	S	5
MC-5	18	144	29	192	S	5
MC-6	19	173	28	298	S	6
MC-7*	19	173	28	298	S	6
Unpaired, T.a. only						
T-1	24		47		S	66
T-2	25		44		S	53
T-3	25		47		S	55
T-4	23		50		L	11
T-5	23		51		L	5
T-6	20		52		L	8
T-7	21		52		L	6
T-8	15		19		S	5
T-9	16		19		S	2
T-10	20		48		L	6
T-11	17		31		S	12
T-12	25		63		S	16

T. antarctica (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. An * indicates experiments where one T.a. container was paired with two P.a. containers. The T.a. is listed twice in those cases.

also resulted in a sheet of *P. antarctica* on the bottom, which would periodically dislodge and fragment. These sheets were screened out with either a 500- or 1000-μm mesh so that only true colonies were used in experiments.

Phytoplankton concentrations and growth rates were calculated from biweekly samples (10–50 μl, taken in duplicate) and from samples taken 1 day prior to the experiment (10–50 μl, five samples per culture). These samples were filtered onto GF/C filters, frozen, and analyzed for chl *a* with a Turner Designs Model AU-005 digital fluorometer and the methods of Smith et al. (1981). *P. antarctica* cultures that were mixtures of single cells and colonies were fractionated with a 20-μm filter prior to filtration to separate single cells (3–8 μm) from colonies. This allowed for measurement of chl *a* present in colonies only versus colonies plus single cells.

Samples for microscopic examination (a few ml per culture) were taken at the same time as for chl *a* determination. Subsamples were transferred with a Hensen–Stempel wide-bore syringe (1.0 ml) to a Sedgewick–Rafter counting chamber. Since time was limited in the field, rough estimates of mean colony size of *P. antarctica* were made by measuring the minimum and maximum dimensions of all colonies within two to five counting chambers, or at least 20 colonies. A determination of mostly single, mixed, and mostly colonial was also made by eye. *T. antarctica* was similarly evaluated for cell diameter and chain length. Cultures were also checked for contamination by other phytoplankton species. Samples (100 ml) were also taken during the experiment (see Section 2.4) and preserved in 0.35% glutaraldehyde/Lugol's solution (Rousseau et al., 1990) for detailed analysis. For *P. antarctica*, cells and colonies were counted. If colonies were present, 100 random colonies were measured at their minimum and maximum dimensions. For *T. antarctica*, cell numbers, dimensions, and number and length of chains were similarly quantified (Table 1).

Finally, cultures were sampled 1 day prior to each experiment for elemental analysis. Four subsamples, each of a different volume, were filtered onto pre-combusted (500 °C, 1 h) A/E filters. These filters were placed into pre-combusted (450 °C, 24 h) aluminum sleeves and plates, stored at 60 °C, 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 used to determine C and N concentrations of a culture. Values of C and N were then used to calculate ratios of C:chl *a*, N:chl *a*, and C:N (Tables 2 and 3).

2.3. Experimental design

Grazing experiments were primarily conducted as paired comparisons between *T. antarctica* and *P. antarctica*. Results of additional, unpaired experiments for *T. antarctica* have been included in some analyses. Phytoplankton concentrations in these experiments were kept below the critical concentration (for both chl *a* and estimated carbon) as defined by Frost (1972), and within the range where clearance rates are at their maximum and relatively constant. This design allowed for paired comparisons of clearance rates even when chl *a* and/or carbon concentrations in the paired containers were similar but not equal. Critical concentrations were estimated from previous studies of grazing by *E. superba* (Price et al., 1988; Ross et al., 1998).

Table 2
Initial values of chl *a*, carbon, C:N and C:chl *a* [experiments are grouped by *P. antarctica* (P.a.) type]

	Chl *a* (μg/l)		Carbon (μg/l)		C:N		C:chl *a*	
	T.a.	P.a.	T.a.	P.a.	T.a.	P.a.	T.a.	P.a.
P.a. as single cells								
S-1	2.6	2.9	101	124	4.3	5.0	40	43
S-2	4.1	4.7	128	313	4.3	4.7	31	66
S-3	5.6	5.1	119	254	4.7	5.1	21	50
S-4	6.8	5.1	162	251	4.7	5.1	24	50
S-5	6.2	5.8	154	248	5.0	5.4	25	43
S-6	6.9	5.8	185	364	4.8	7.4	27	63
P.a. as small colonies								
SC-1	2.9	2.8	134	344	10.1	6.6	46	125
SC-2	4.6	3.7	114	165	4.0	4.5	25	45
SC-3	3.5	3.9	92	216	4.4	5.4	26	55
SC-4	5.0	4.1	121	383	4.6	6.2	24	94
SC-5	6.5	5.7	155	456	4.8	6.7	24	80
P.a. as medium colonies								
MC-1	17.4	11.4	458	884	3.9	5.2	26	78
MC-2	17.9	4.5	501	552	4.4	14.0	28	124
MC-3	8.2	3.3	226	415	4.2	15.2	27	126
MC-4	8.2	3.0	226	196	4.2	6.0	27	65
MC-5	10.8	2.6	297	172	4.2	6.0	27	65
MC-6	9.5	7.8	231	517	4.3	5.8	24	67
MC-7*	9.5	3.6	231	241	4.3	5.8	24	67
Unpaired, T.a. only								
T-1	4.7		119		4.4		25	
T-2	8.3		239		5.2		29	
T-3	5.8		174		4.7		30	
T-4	6.8		333		4.6		49	
T-5	4.9		110		4.9		22	
T-6	6.2		153		4.4		25	
T-7	5.4		131		4.3		25	
T-8	5.6		209		6.3		38	
T-9	6.9		206		5.3		30	
T-10	5.4		122		4.8		23	
T-11	20.1		525		3.7		26	
T-12	10.6		518		5.1		49	

A related question was which phytoplankton variable to choose as the standard for the different phytoplankton species. Carbon is roughly proportional to the total mass/energy available, and also appears to be relevant 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. For experiments with single cell and small, colonial *P. antarctica*, target chl *a* concentrations were equal for the paired containers. However, analyses from these experiments showed that C:chl *a* ratios for *P. antarctica* were approximately double the C:chl *a* ratios for *T.*

Table 3
C:N and C:chl *a* statistics for each *P. antarctica* (P.a.) category, and for *T. antarctica* (T.a.)

	C:N			C:chl *a*		
	Mean	± S.D.	C.V.	Mean	± S.D.	C.V.
P.a., single cells	5.38	± 0.89	0.16	50.9	± 10.0	0.16
P.a., small colonies	5.86	± 0.94	0.16	79.7	± 31.9	0.40
P.a., medium colonies	8.34	± 4.29	0.51	99.7	± 42.0	0.42
T.a., all	4.61	± 0.51	0.24	29.2	± 7.8	0.27

antarctica (Tables 2 and 3). 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 with medium colonies were planned as half the values for *T. antarctica* treatments.

2.4. Experimental set-up and sampling

Experimental containers (47-cm diameter × 53-cm height) were filled with 40–45 l of filtered (0.45 μm) seawater and allowed approximately 24 h for temperature equilibration prior to the experiment. The volume of phytoplankton required for each container to achieve the desired concentrations, as calculated from the chl *a* measurements and the estimated growth rate of each culture, was added to the containers approximately 10 h prior to the experiment. Additional filtered seawater was then added to the containers to bring the volumes up to approximately 55 l. The experimental vessels were then mixed continuously with a plunger-type stirring system modified from Frost (1972). After the phytoplankton was added, the experimental vessels were left overnight to equilibrate to experimental temperature and light conditions (0.5 ± 0.3 °C, dim light). Simultaneously, krill for the experiments were moved into the experimental room and acclimated overnight to the environmental conditions in two 20-l buckets of filtered seawater.

Each experiment lasted 12 h, divided into two consecutive feeding periods of 6 h each. Identical, replicate containers were used for each feeding period, making four containers total for each paired experiment. During the first 6-h period, the phytoplankton concentration in the two containers without krill was monitored as the control vessels. Krill (9–30 per container) were transferred individually, with a taut net, to these vessels for the second feeding period.

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. During the second 6-h 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 for microscopic analysis (see Section 2.2). Krill were measured and individually weighed after the experiment.

For some experiments with medium *P. antarctica* colonies, there was only one pair of containers and only a single feeding period, due to the difficulty of growing this type of *P. antarctica*. 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.

2.5. Grazing rate calculations

Phytoplankton growth coefficients (k) and loss coefficients (b), as defined by Frost (1972), were calculated with best fit exponential regressions of time (t) and chl a concentration. The coefficients are the exponents of these regressions. Control containers with no krill were used to calculate k, while experimental containers with krill were used to calculate b. The chl a value used was the average of the five replicates taken at each time period. The grazing coefficient (g) was the difference between k and b (Frost, 1972). In most cases, $k=0$ in the control containers. We assumed this would also be true for the experimental containers, given the dim light conditions in the experimental room.

Grazing coefficients were converted to clearance rates (F in l·g wet $wt^{-1}\cdot h^{-1}$), i.e. the wet-weight specific rate at which a given volume of seawater is swept clear of phytoplankton by krill (Frost, 1972). Carbon ingestion rates (I_C) and nitrogen ingestion rates (I_N) were then calculated from 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:

$$I_C = F \times [\text{chl } a]_0 \times (\text{C:chl } a)$$
$$I_N = F \times [\text{chl } a]_0 \times (\text{N:chl } a).$$

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 the paired comparisons of medium *P. antarctica* and *T. antarctica* ($n=7$ for each phytoplankton type). Clearance rates (F) were calculated for each 2-h interval during the first feeding period, and clearance rates among the three intervals were compared with a Friedman two-way ANOVA.

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).

3. Results

Results are presented as follows: (1) preliminary tests to determine the most valid approach to analysis of the experimental results; (2) pairwise comparisons of clearance rates between species; (3) variability of clearance rates within species; and (4) comparisons of carbon and nitrogen-based ingestion rates between and within species.

3.1. Preliminary tests

Clearance rates for the first and second feeding periods of each experiment, grouped by phytoplankton species, were compared with a Wilcoxon signed rank test to determine whether the two periods could be treated as replicate experiments. Clearance rates by *E. superba* on both *P. antarctica* and *T. antarctica* were significantly higher for the first feeding period, when the krill had been starved for the previous 10 h, than the second feeding period, when krill had been acclimated to food conditions for 6 h (*P. antarctica*: 64% higher on average, $p = 0.002$; *T. antarctica*: 43% higher on average, $p = 0.035$). The first feeding period was used in most further analyses, since some experiments included only the first feeding period (see Methods). Also, results for the second feeding period were qualitatively consistent with those from the first feeding period for paired experiments with *T. antarctica* and both single cells and medium colonies of *P. antarctica* (Haberman, unpublished data). However, data from both the first and second feeding periods are presented for experiments which included small *P. antarctica* colonies because statistical analyses of the first and second feeding periods led to different conclusions.

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. For the paired comparisons with medium *P. antarctica* ($n = 7$), clearance rates for the three 2-h intervals within the first feeding period were available. Clearance rates among the three 2-h intervals were not significantly different from each other for either *P. antarctica* (Friedman two-way ANOVA, $p = 0.15$) or *T. antarctica* ($p = 1.0$). Therefore, the entire 6-h period was appropriate to use for comparing clearance rates in these experiments.

Clearance rates were also analyzed as a function of initial carbon concentration to test the assumption that initial phytoplankton concentrations were below the critical concentration. These tests were necessary since carbon was not always equal for the two phytoplankton types in the paired treatments. Treatments were grouped into four phytoplankton types: single cell, small colonies and medium colonies of *P. antarctica*, and all *T. antarctica*.

Clearance rates were not a function of initial carbon concentration for any of size categories of *P. antarctica* nor for *T. antarctica* (regression analysis, $p > 0.20$ for all groups). These results support the assumption that initial chl *a* concentrations were below the critical concentration for all experiments, and that further comparisons are valid even for experiments which were not matched for carbon concentration.

3.2. Pairwise comparisons of clearance rates between species

Experiments were analyzed as paired comparisons of clearance rates by *E. superba* on *P. antarctica* and *T. antarctica*. Experimental pairs were grouped by size category of *P. antarctica* (small colonies, medium colonies, and single cells). These groupings also separated *P. antarctica* according to C:N and C:chl *a*. No significant differences were found between single cells of *P. antarctica* and *T. antarctica* for C:N (Tukey HSD multiple comparisons, $p = 0.72$) or C:chl *a* ($p = 0.06$). In contrast, C:chl *a* was significantly different between small colonies of *P. antarctica* and *T. antarctica* ($p < 0.001$). C:N was not significantly different for this pairing ($p = 0.72$). Medium

colonies of *P. antarctica* differed significantly from *T. antarctica* for both C:chl *a* and C:N ($p < 0.001$).

Differences in clearance rates between *T. antarctica* and *P. antarctica* were tested with a Wilcoxon signed rank test; $p < 0.05$ was the accepted significance level. Clearance rates by krill on single cell *P. antarctica* were nearly an order of magnitude lower than on *T. antarctica* (Fig. 1a; Table 4). Clearance rates by *E. superba* on small *P. antarctica* colonies were also lower than on *T. antarctica* in four out of five pairings for the first feeding period (Fig. 1b). The mean clearance rate for *P. antarctica* was approximately 70% of the mean for *T. antarctica*, but this difference was not significant (Table 4). However, during the second feeding period of the same paired experiments, clearance by krill on *P. antarctica* colonies was lower than on *T. antarctica* in all pairings (Fig. 1c), with a significantly lower mean rate for *P. antarctica*, approximately 35% that for *T. antarctica* (Table 4). Clearance rates by krill on medium *P. antarctica* colonies were significantly less than on *T. antarctica*, with the mean clearance rate for *P. antarctica* less than 30% of the rate for *T. antarctica* (Fig. 1d; Table 4).

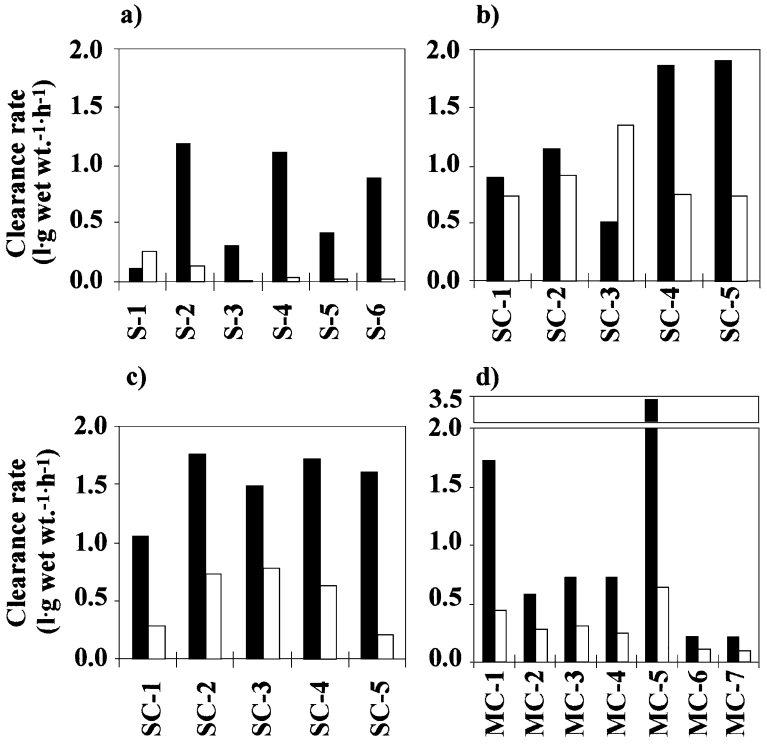

Fig. 1. Paired comparisons of clearance rates (l·g wet $wt^{-1} \cdot h^{-1}$) between *T. antarctica* (black bars) and small *P. antarctica* colonies (white bars) for: (a) single cell *P. antarctica*, first feeding period; (b) small *P. antarctica* colonies, first feeding period; (c) small *P. antarctica* colonies, second feeding period; (d) medium *P. antarctica* colonies, first feeding period.

Table 4
Clearance rate (l·g wet wt^{-1}·h^{-1}) values and statistics for paired experiments

P.a. type	Total pairs	Period	T.a.		P.a.		WSRT
			Mean	S.E.	Mean	S.E.	*p*-value
Single cell	6	1	0.68	0.18	0.09	0.04	0.03*
Small colonies	5	1	1.27	0.28	0.91	0.12	0.23
Small colonies	5	2	1.54	0.28	0.53	0.26	0.04*
Medium colonies	7	1	1.10	0.44	0.30	0.07	0.02*

WSRT: *p*-values for Wilcoxon's signed ranked test; an * indicates a significant difference ($p < 0.05$) in clearance rates. All significant values indicate a lower clearance rate for P.a. See text and Table 1 for further size information and abbreviations.

3.3. Variability of clearance rates within species

Clearance rates by *E. superba* on small *P. antarctica* colonies were significantly greater than clearance rates on both medium colonies and single cells of *P. antarctica* (Tukey HSD multiple comparisons, $p < 0.001$). 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 *P. antarctica* (Fig. 2). Clearance rate significantly decreased with increasing colony size ($y = -0.004x + 1.11$, $r^2 = 0.69$, $p < 0.001$). Since *Phaeocystis* colony size is sometimes correlated with C:N and/or C:chl *a* (Davies et al., 1992), the relationship between colony size and clearance rate may be confounded by these factors. However, a regression analysis found no significant correlation between colony size and either C:N ($p = 0.63$) or C:chl *a* ($p = 0.70$).

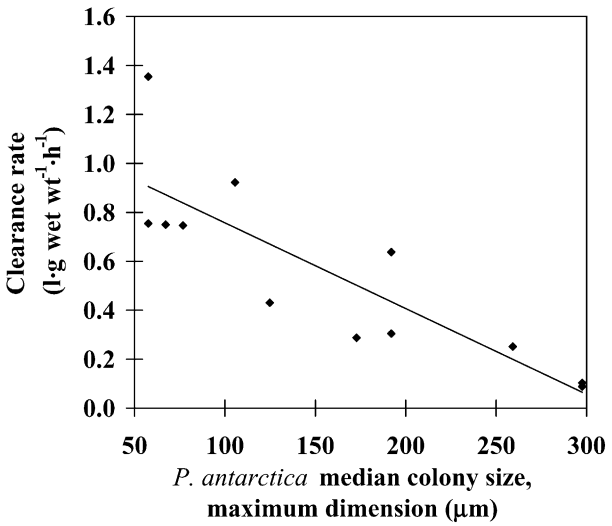

Fig. 2. Clearance rates (l·g wet wt^{-1}·h^{-1}) for *E. superba* on *P. antarctica* as a function of initial colony size (µm); $y = -0.004x + 1.11$, $r^2 = 0.69$, $p < 0.001$.

Although *T. antarctica* cultures were grown under similar conditions for all experiments, cell diameter, degree of chain formation, minimum and maximum dimensions of particles, specific growth rates, and C:N ratios varied. Cell diameter was bimodal, with either "small" cells (20–30 μm) or "large" cells (48–60 μm) dominating an experiment (Table 1). Chain formation was relatively rare for large cells; fewer than 11% of large cells were found in chains for any experiment. In contrast, chain formation varied widely for small cells, with 0–83% of the cells found in chains (Table 1). Clearance rates of large cells were in the same range as small cells with low rates of chain formation (Fig. 3). However, for small cells, clearance rate was significantly correlated with the percent of cells found in chains (Fig. 3; $r^2 = 0.49$, $p = 0.0004$). No other significant relationships were found ($p > 0.14$ for all regressions). Note that the clearance rate for *T. antarctica* obtained in Experiment MC-5 was an outlier (more than double the average clearance rate), and was not included in this, or subsequent, analyses.

3.4. Carbon- and nitrogen-based ingestion rates

Carbon and nitrogen ingestion rates during the first feeding period for each size category of *P. antarctica* and *T. antarctica* were analyzed as functions of initial carbon concentration (Fig. 4). Neither carbon (Fig. 4a) nor nitrogen (Fig. 4b) ingestion rates for *P. antarctica* were significantly correlated with initial carbon concentration for any size category of *P. antarctica* (regression analysis, $p \geq 0.12$ for carbon ingestion and $p > 0.50$ for nitrogen ingestion) Both carbon and nitrogen ingestion rates for small *P. antarctica* colonies were significantly higher than for either medium *P. antarctica* colonies (Tukey HSD multiple comparisons, $p \leq 0.03$) or single cell *P. antarctica* ($p \leq 0.001$). Note that

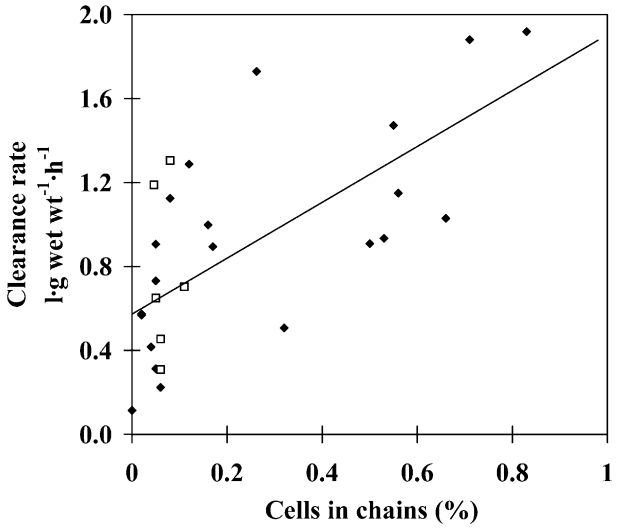

Fig. 3. Clearance rates (l·g wet wt^{-1}·h^{-1}) for *E. superba* on *T. antarctica* as a function of the percent of *T. antarctica* particles in chains of two or more cells. Filled diamonds: small cells; open squares: large cells. Regression is for small cells only; $y = 1.33x + 0.49$, $r^2 = 0.56$, $p = 0.0004$.

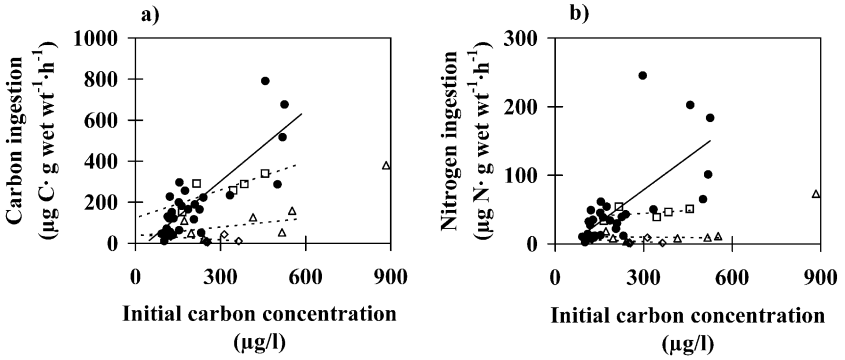

Fig. 4. Ingestion rates of carbon and nitrogen by *E. superba* grazing on *P. antarctica* (P.a.) and *T. antarctica* (T.a.), plotted as a function of initial carbon concentration ($\mu g \cdot l^{-1}$). (a) Carbon ingestion versus initial carbon concentration ($\mu g \cdot l^{-1}$). Open diamonds: P.a. as single cells; open squares: P.a. as small colonies; open triangles: P.a. as medium colonies; closed circles: T.a. Solid regression line is for *T. antarctica* ($y = 1.15x - 42$, $r^2 = 0.67$, $p < 0.001$). Dotted lines are regressions (nonsignificant) for each of the three P.a. types. (b) Nitrogen ingestion versus initial carbon concentration ($\mu g \cdot l^{-1}$). Regression line is for *T. antarctica* ($y = 0.29x - 15.8$, $r^2 = 0.63$, $p < 0.001$). Symbols as above.

the outlier (Experiment MC-1) is not included in the regression analyses because initial carbon concentrations were out of range.

Carbon and nitrogen ingestion rates for *E. superba* on *T. antarctica* were both significantly correlated with initial carbon concentration ($r^2 = 0.67$, $p < 0.001$, Fig. 4). While carbon and nitrogen ingestion rates for all size categories of *P. antarctica* overlap with *T. antarctica* rates at low initial carbon concentrations, 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.

4. Discussion

The strength of the experimental design is that grazing on the two phytoplankton species can be directly compared, minimizing the confounding effects of krill feeding history, time of year, interannual differences in culture status, and subtle changes in experimental conditions that could contribute to variability in feeding rates unrelated to the phytoplankton type. These comparisons showed that clearance rates on *P. antarctica* were (1) generally lower than on *T. antarctica*, and (2) dependent upon size of the "particle" in the culture. Also, krill cleared and ingested small colonies of *P. antarctica* at significantly higher rates than either medium colonies or single cells.

Variability in grazing rates seen in this study may reflect the physical differences within and between the two phytoplankton species. For example, differences in grazing rates among the three types of *P. antarctica* suggest that there is optimal particle size which is best grazed by *E. superba*. At the low end of the size range,

single cells may be too small to be effectively grazed. Previous studies suggest a reduced sieving efficiency of *E. superba*'s feeding basket for particles <10 μm (McClatchie and Boyd, 1983; Quetin and Ross, 1985; Ishii et al., 1985). At the upper end of the size range, the negative relationship of clearance rate and colony size (Fig. 2) suggests that relatively low grazing rates by krill on medium *P. antarctica* may be due to their larger size. However, krill are capable of ingesting much larger particles. For example, Price et al. (1988) showed successful grazing by krill on copepods larger than the colonial *P. antarctica* used in this study. When preying on copepods, the krill appeared to feed raptorially, not by compression-filtration. Possibly, the medium colonies were too large for efficient capture by filtration, but too small for efficient raptorial capture. Experiments with larger *P. antarctica* colonies could be used to test this hypothesis.

Another physical factor that could affect feeding is the stickiness of the mucopolysaccharides, which form the matrix of *P. antarctica* colonies. It is possible that the medium *P. antarctica* colonies clogged the krill's feeding basket, as has been found for other filter-feeding invertebrates (Pieters et al., 1980; Schnack et al., 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 that might also interfere with feeding. Recent studies suggest that TEP production is associated with larger *P. antarctica* colonies (Hong et al., 1997).

Physical factors also appeared to affect grazing by krill on *T. antarctica*. Specifically, chain formation contributed significantly to this variability when small cells were grazed. 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, 1983). This result is also consistent with the relationships between size and clearance found in other studies (Quetin and Ross, 1985). However, there was no difference between clearance rates for single *T. antarctica* cells of different sizes, as would be expected if size was the key factor.

Clearance rates on small *P. antarctica* were similar to *T. antarctica* for the first feeding period, and both carbon and nitrogen ingestion rates overlapped for these two groups of phytoplankton when comparisons across experiments were made (Fig. 4). This suggests that under certain conditions, intake of *P. antarctica* could be equal to intake of *T. antarctica*. However, the nutritional value of *P. antarctica* is likely to be inferior to *T. antarctica* because, based on C:chl *a* ratios of small colonies versus single cells (Table 3), approximately 40% of the carbon within small colonies is extracellular, and composed of mucopolysaccharides. This form of carbon is probably low in nutritional value compared to diatom carbon (Sargent et al., 1985).

C:N ratios are potential indicators of food quality for krill (Frazer et al., 1997). C:N was not strictly tied to colony size for *P. antarctica*; however, the relatively large coefficient of variation of C:N ratios for medium colonies suggests that their quality was more variable than small colonies. In the field, C:N ratios of *Phaeocystis* spp. blooms often increase when large colonies predominate (Davies et al., 1992). If the nutritional value of larger *P. antarctica* colonies is more variable than small colonies, it may be advantageous for the krill to limit their intake of larger *P. antarctica* and

instead search for more consistently nutritional food resources, as optimal foraging theory would predict (MacArthur and Pianka, 1966).

These studies only compared grazing rates among monocultures of the two phytoplankton species. Assimilation and growth studies would help elucidate the ability for *P. antarctica* carbon, nitrogen, and lipids to be effectively utilized by krill. Also, grazing experiments carried out with mixtures of *P. antarctica* and *T. antarctica* would clarify whether krill select *T. antarctica* over *P. antarctica* (Haberman et al., 2002a).

5. Conclusions

Consistent and significant differences between grazing rates by krill on *P. antarctica* and *T. antarctica*, as well as among the different size classes of *P. antarctica*, were found in this study. These differences were detected against a background of high variability in grazing rates on *T. antarctica*. Grazing on *P. antarctica* was dependent upon size: medium colonies and single cells of *P. antarctica* were cleared and ingested at relatively low rates compared to *T. antarctica* and small colonies of *P. antarctica*. Clearance and ingestion rates on small colonies of *P. antarctica* overlapped those of *T. antarctica.* 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 *P. 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 *P. antarctica* as a food source for Antarctic krill.

Acknowledgements

This research was supported by National Science Foundation grants OPP90-11927 and OPP96-32763 to the Palmer LTER. Additional funds were provided by the Office of Research and the Marine Science Institute of the University of California at Santa Barbara. This project benefited from the hard work of many people. In particular, Tim Newberger, Janice Jones, Tracy Shaw, and many volunteers provided exceptional assistance. We also thank Captain Karl Sanden and the crew of the M/V Polar Duke. Finally, Mark Melcon, Dave Vella, Marian Moyher, and numerous other Antarctic Support Associates at Palmer Station must be acknowledged for their excellent support. This is Palmer LTER Contribution No. 219. **[RW]**

References

Alldredge, A.L., Passow, U., Logan, B., 1993. The abundance and significance of a class of large transparent organic particles in the ocean. Deep-Sea Res. 40, 1131–1140.

Atkinson, A., Snÿder, A., 1997. Krill–copepod interactions at South Georgia, Antarctica: I. Omnivory by *Euphausia superba*. Mar. Ecol., Prog. Ser. 160, 63–76.

Bidigare, R.R., Iriarte, J.L., Kang, S.-H., Karentz, D., Ondrusek, M.E., Fryxell, G.A., 1996. Phytoplankton: quantitative and qualitative assessments. In: Ross, R.M., Hofmann, E.E., Quetin, L.B. (Eds.), Foundations for Ecological Research West of the Antarctic Peninsula. American Geophysical Union, Washington, DC, pp. 173–198.

Davidson, A.T., Marchant, J.J., 1987. Binding of manganese by Antarctic *Phaeocystis pouchetii* and the role of bacteria in its release. Mar. Biol. 95, 481–487.

Davidson, A.T., Marchant, H.J., 1992. The biology and ecology of *Phaeocystis* (Prymnesiophyceae). Prog. Phycol. Res. 8, 1–45.

Davies, A.G., De Madariago, I., Bautista, B., Fernandez, E., Harbour, D.S., Serret, P., et al., 1992. The ecology of a coastal *Phaeocystis* bloom in the north-western English Channel in 1990. J. Mar. Biol. Assoc. U.K. 72, 691–708.

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

Frazer, T.K., Ross, R.M., Quetin, L.B., Montoya, J.P., 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: a stable isotope approach. J. Exp. Mar. Biol. Ecol. (212), 259–275.

Frost, B.W., 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 17 (6), 805–815.

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

Guillard, R.R.L., Ryther, J.H., 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol. 8, 229–245.

Haberman, K.L., Ross, R.M., Quetin, L.B., 2002a. Diet of the Antarctic krill (*Euphausia superba* Dana): II. Selective grazing in mixed phytoplankton assemblages. J. Exp. Mar. Biol. Ecol. 283, 97–113.

Haberman, K.L., Ross, R.M., Quetin, L.B., Vernet, M., Nevitt, G.A., Kozlowski, W., 2002b. Grazing by Antarctic krill *Euphausia superba* on *Phaeocystis antarctica*: an immunochemical approach. Mar. Ecol., Prog. Ser. 141, 139–149.

Hamner, W.M., 1988. Biomechanics of filter feeding in the Antarctic krill *Euphausia superba*: review of past work and new observations. J. Crustac. Biol. 8 (2), 149–163.

Hansen, B., Verity, P., Falkenhaug, T., Tande, K.S., Norrbin, F., 1994. On the trophic fate of *Phaeocystis pouchetii* (Harriot): V. Trophic relationships between *Phaeocystis* and zooplankton: an assessment of methods and size dependence. J. Plankton Res. 16 (5), 487–511.

Hong, Y., Smith, W.O.J., White, A.-M., 1997. Studies on transparent exopolymer particles (TEP) produced in the Ross Sea (Antarctica) and by *Phaeocystis antarctica*. J. Phycol. 33 (3), 368–376.

Ishii, H., Omori, M., Murano, M., 1985. Feeding behavior of the Antarctic krill *E. superba* Dana: I. Reaction to size and concentration of food particles. Trans. Tokyo Univ. Fish. 6, 117–124.

Lascara, C., Hofmann, E., Ross, R., Quetin, L., 1999. Seasonal variability in the distribution of Antarctic krill, *Euphausia superba*, west of the Antarctic Peninsula. Deep-Sea Res. 46 (6), 951–984.

MacArthur, R.H., Pianka, E.R., 1966. On optimal use of a patchy environment. Am. Nat. 100 (916), 603–609.

Marin, V., Huntley, M.E., Frost, B., 1986. Measuring feeding rates of pelagic herbivores: analysis of experimental design and methods. Mar. Biol. 93, 49–58.

McClatchie, S., Boyd, C.M., 1983. Morphological study of sieve efficiencies and mandibular surfaces in the Antarctic krill, *Euphausia superba*. Can. J. Fish. Aquat. Sci. 40, 955–967.

Meyer, M.A., El-Sayed, S.Z., 1983. Grazing of *Euphausia superba* Dana on natural phytoplankton populations. Polar Biol. 1, 193–197.

Miller, D.G.M., Hampton, I., 1989. Biology and ecology of the Antarctic krill (*Euphausia superba* Dana). BIOMASS Sci. Ser. 9, 1–166.

Moline, M.M., Prézelin, B.B., 1996. Long-term monitoring and analyses of physical factors regulating variability

in coastal Antarctic phytoplankton biomass, in situ productivity and taxonomic composition over subseasonal, seasonal and interannual time scales. Mar. Ecol., Prog. Ser. 145, 143–160.

Pakhomov, E.A., Perissinotto, R., Froneman, P.W., Miller, D.G.M., 1997. Energetics and feeding dynamics of *Euphausia superba* in the South Georgia region during the summer of 1994. J. Plankton Res. 19, 399–423.

Pieters, H., Kluytmans, J.H., Zandee, D.I., Cadee, G.C., 1980. Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. Neth. J. Sea Res. 14, 349–361.

Price, H.J., Boyd, K.R., Boyd, C.M., 1988. Omnivorous feeding behavior of the Antarctic krill *Euphausia superba*. Mar. Biol. 97, 67–77.

Quetin, L.B., Ross, R.M., 1985. Feeding by Antarctic krill, *Euphausia superba*: does size matter? In: Siegfried, W.R., Condy, P.R., Laws, R.M. (Eds.), Antarctic Nutrient Cycles and Food Webs. Springer-Verlag, Berlin, pp. 372–377.

Quetin, L.B., Ross, R.M., 1991. Behavioral and physiological characteristics of the Antarctic krill, *Euphausia superba*. Am. Zool. 31 (1), 49–63.

Quetin, L.B., Ross, R.M., Frazer, T.K., Haberman, K.L., 1996. Factors affecting the distribution and abundance of zooplankton, with an emphasis on the Antarctic krill, *Euphausia superba*. In: Ross, R.M., Hofmann, E.E., Quetin, L.B. (Eds.), Foundations for Ecological Research West of the Antarctic Peninsula. American Geophysical Union, Washington, DC, pp. 357–371.

Ross, R.M., Quetin, L.B., 1986. How productive are Antarctic krill? Biosci. J. 36 (4), 264–269.

Ross, R.M., Quetin, L.B., Lascara, C.M., 1996. Distribution of Antarctic krill and dominant zooplankton west of the Antarctic Peninsula. In: Ross, R.M., Hofmann, E.E., Quetin, L.B. (Eds.), Foundations for Ecological Research West of the Antarctic Peninsula. American Geophysical Union, Washington, DC, pp. 173–198.

Ross, R.M., Quetin, L.B., Haberman, K.L., 1998. Interannual and seasonal variability in short-term grazing impact of *Euphausia superba* in nearshore and offshore waters west of the Antarctic Peninsula. J. Mar. Syst. 17, 261–273.

Ross, R.M., Quetin, L.B., Baker, K.S., Vernet, M., Smith, R.C., 2000. Growth limitation in young *Euphausia superba* under field conditions. Limnol. Oceanogr. 45 (1), 31–43.

Rousseau, V., Mathot, S., Lancelot, C., 1990. Calculating carbon biomass of *Phaeocystis* sp. from microscopic observations. Mar. Biol. 107, 305–314.

Sargent, J.R., Eilertsen, H.C., Falk-Petersen, S., Taasen, J.P., 1985. Carbon assimilation and lipid production in phytoplankton in the northern Norwegian fjords. Mar. Biol. 85, 109–116.

Schnack, S.B., Smetacek, V., Bodungen, V.B., Stegmann, P., 1985. Utilization of phytoplankton by copepods in Antarctic waters during spring. 18th European Marine Biology Symposium, Oslo. Wiley, Chichester, pp. 65–79.

Sieburth, J.M., 1960. Acrylic acid, an 'antibiotic' principle in *Phaeocystis* in Antarctic waters. Science 132, 676–677.

Smith, R.C., Baker, K.F., Dustan, P., 1981. Fluorometer techniques for measurement of oceanic chlorophyll in the support of remote sensing. Scripps Institute of Oceanography, University of California at San Diego.

Stuart, V., 1989. Observations on the feeding of *Euphausia lucens* on natural phytoplankton suspensions in the southern Benguela upwelling region. Cont. Shelf Res. 9 (11), 1017–1028.

Veldhuis, M.J.W., Admiraal, W., 1987. Influence of phosphate depletion on the growth and colony formation of *Phaeocystis pouchetii*. Mar. Biol. 95, 47–54.

Verity, P.G., Villareal, T.A., Smayda, T.J., 1988. Ecological investigations of blooms of colonial *Phaeocystis pouchetii*: I. Abundance, biochemical composition, and metabolic rates. J. Plankton Res. 10 (2), 219–248.

Virtue, P., Nichols, P.D., Nicol, S., McMinn, A., Sikes, E.L., 1993. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarct. Sci. 5 (2), 169–177.

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